Figure 1.

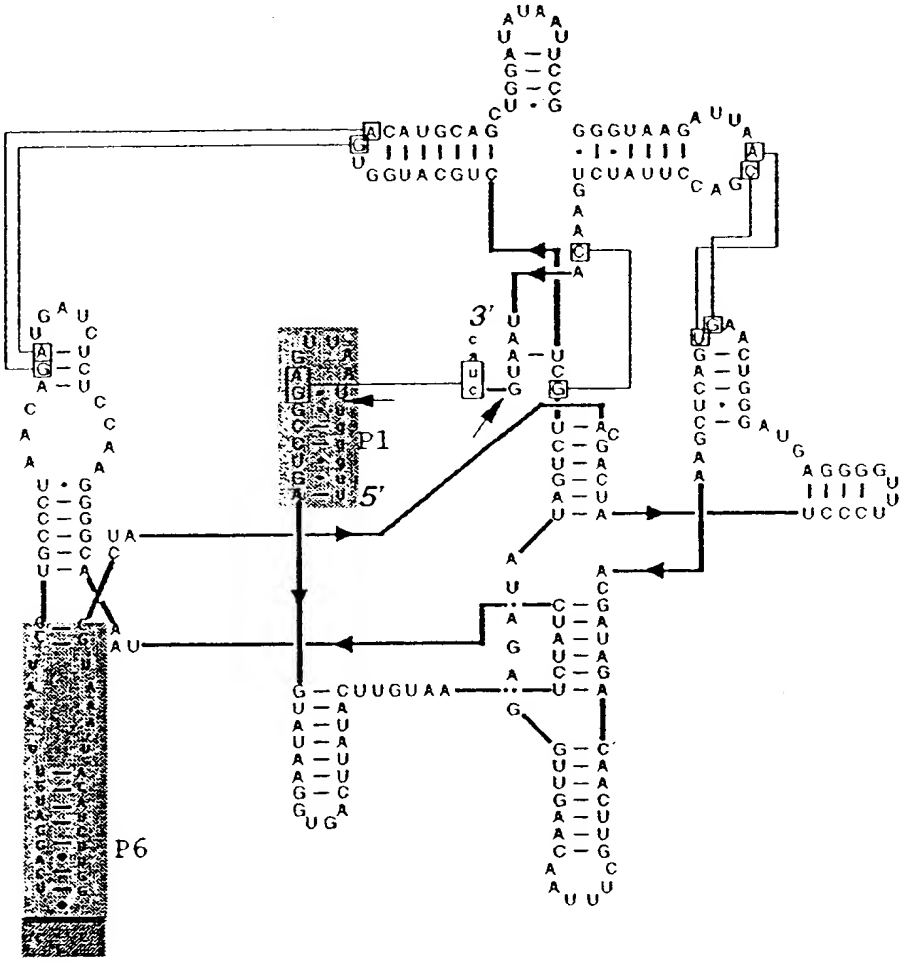
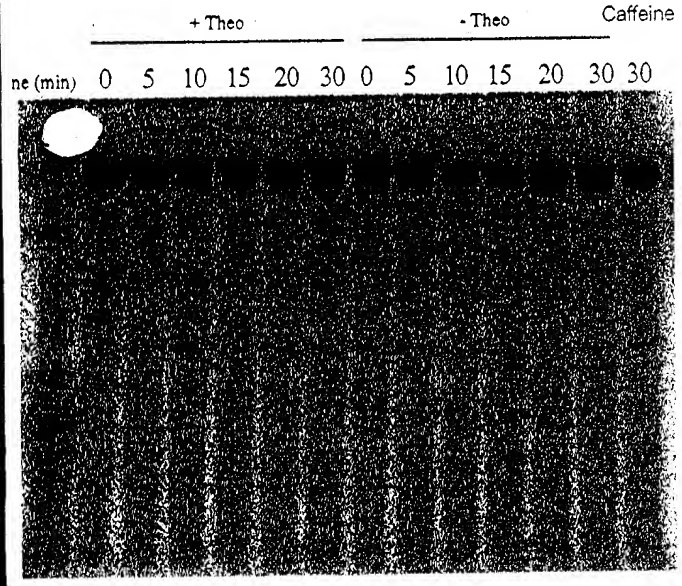
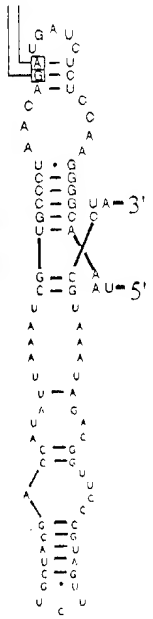


Figure 2a.





GpIThiP6.131

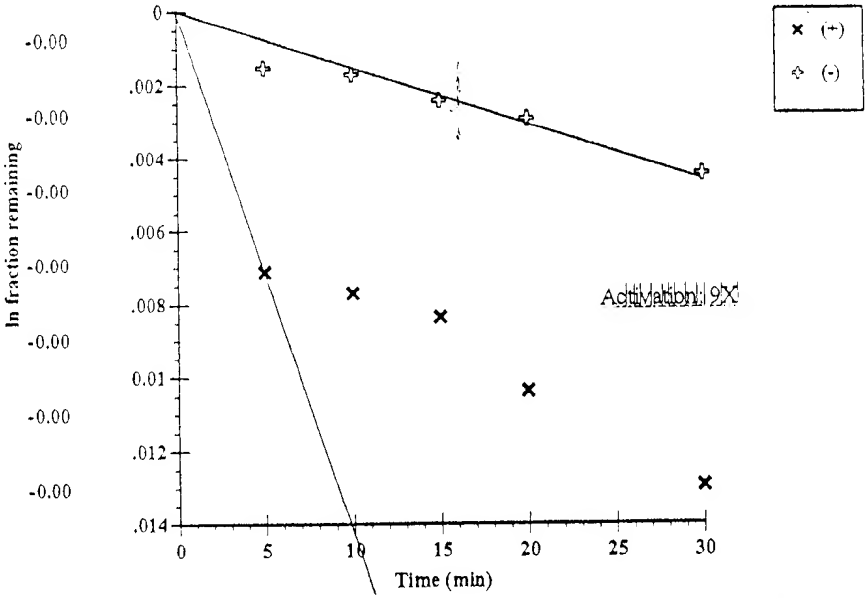
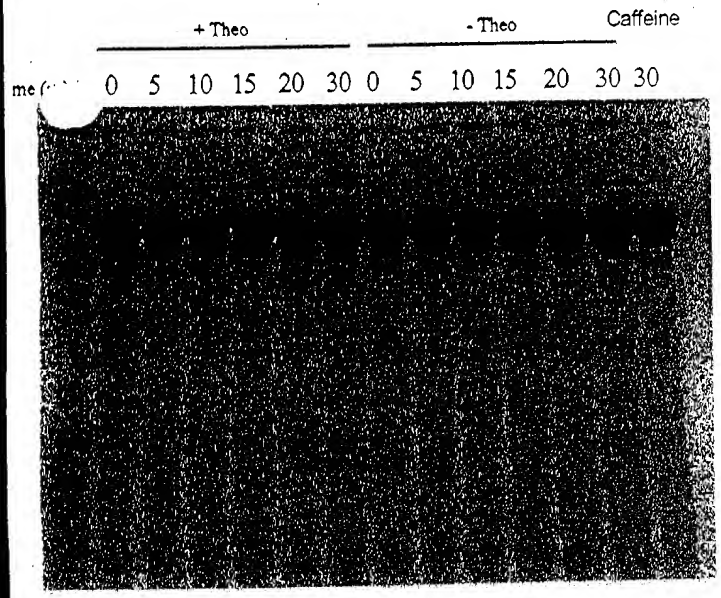
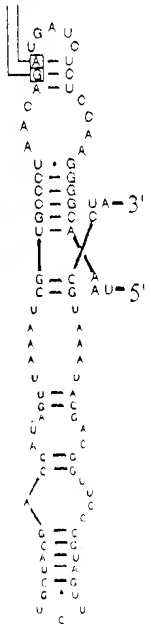
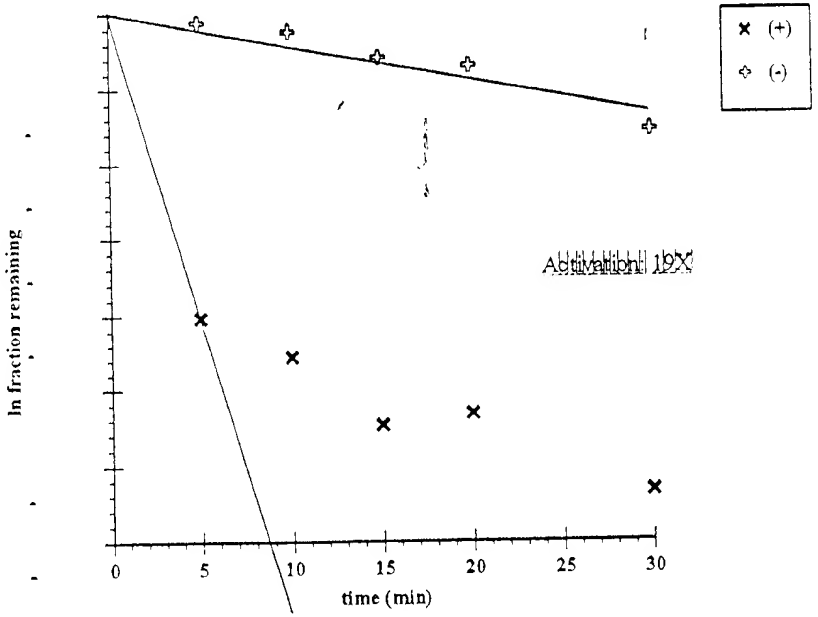


Figure 2b.



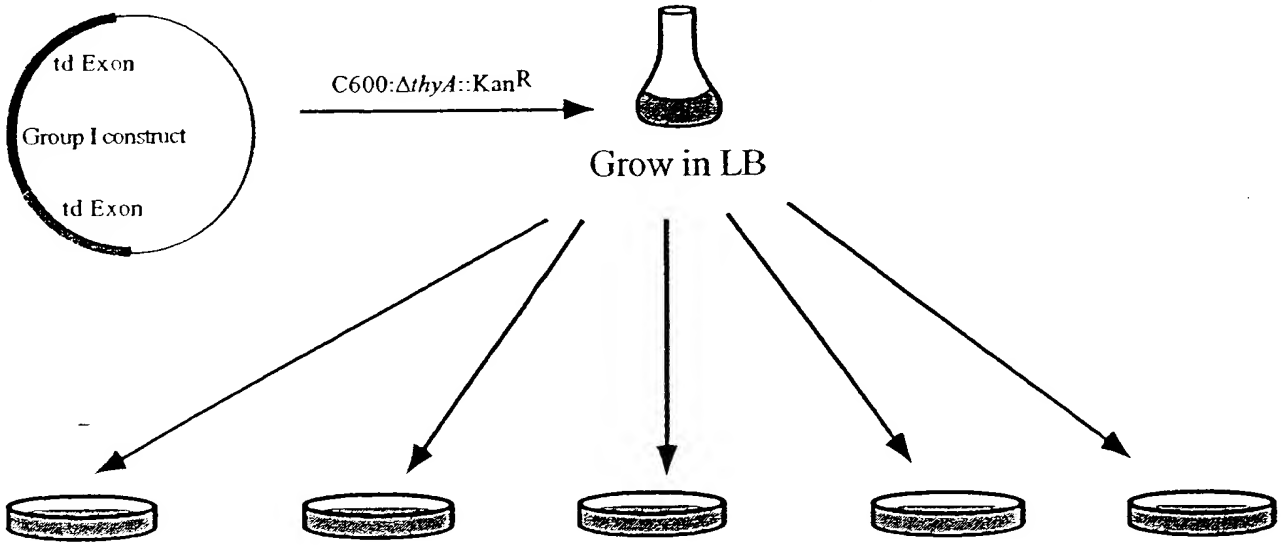




GpITh2P6.133

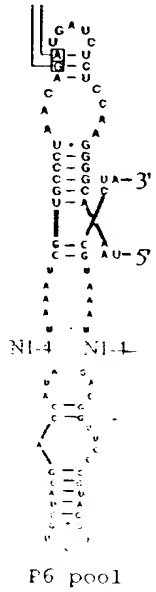
Figure 3.

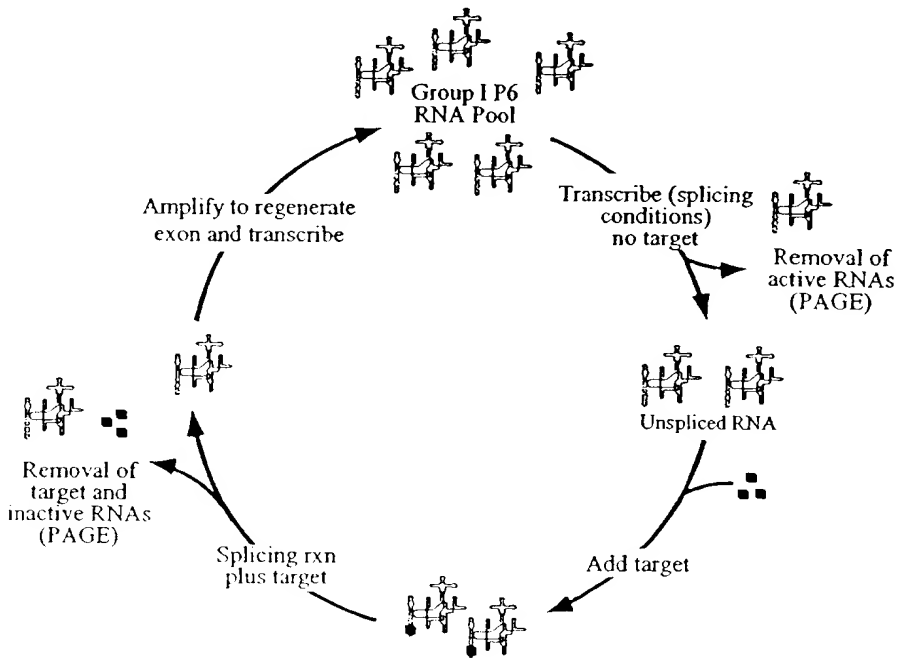
In Vivo Assay

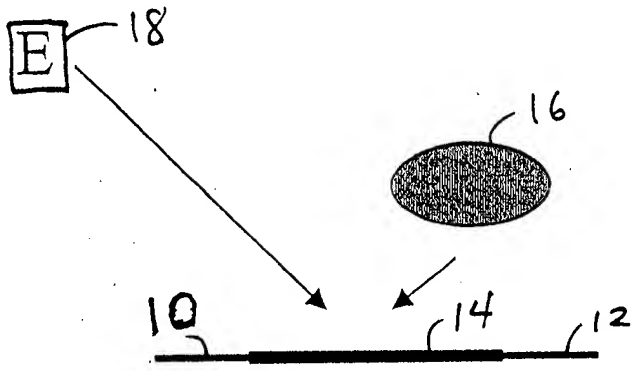


Media	+Thymine	+Thymine + Trimethoprim + Theo	+Thymine + Trimethoprim	-Thymine + Theo	-Thymine
inactive aptazyme phenotype	TS-	TS-	TS-	TS-	TS-
active aptazyme phenotype	TS-	TS+	TS-	TS+	TS-

Figure 4.


3'
5'
NI-4
NI-1
P6 pool


Group I P6
RNA Pool
Transcribe (splicing
conditions)
no target
Removal of
active RNAs
(PAGE)
Unspliced RNA
Add target
Splicing rxn
plus target
Removal of
target and
inactive RNAs
(PAGE)
Amplify to regenerate
exon and transcribe



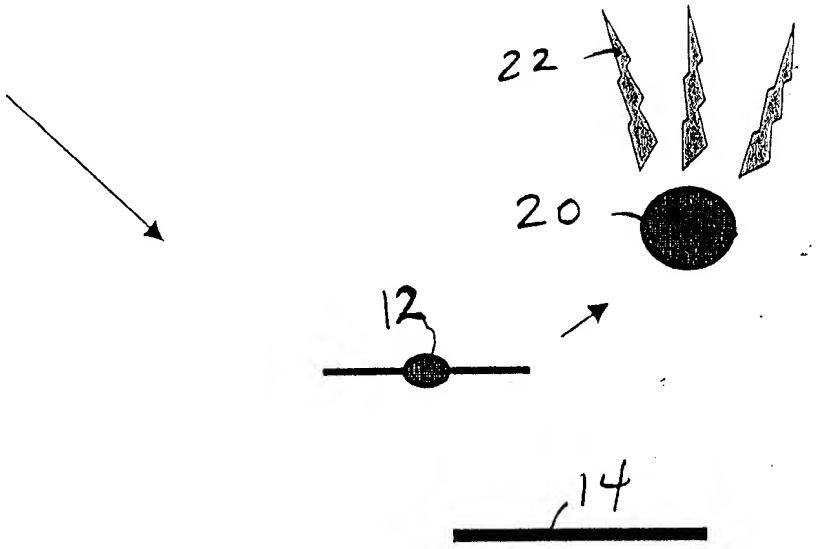


Fig. 5

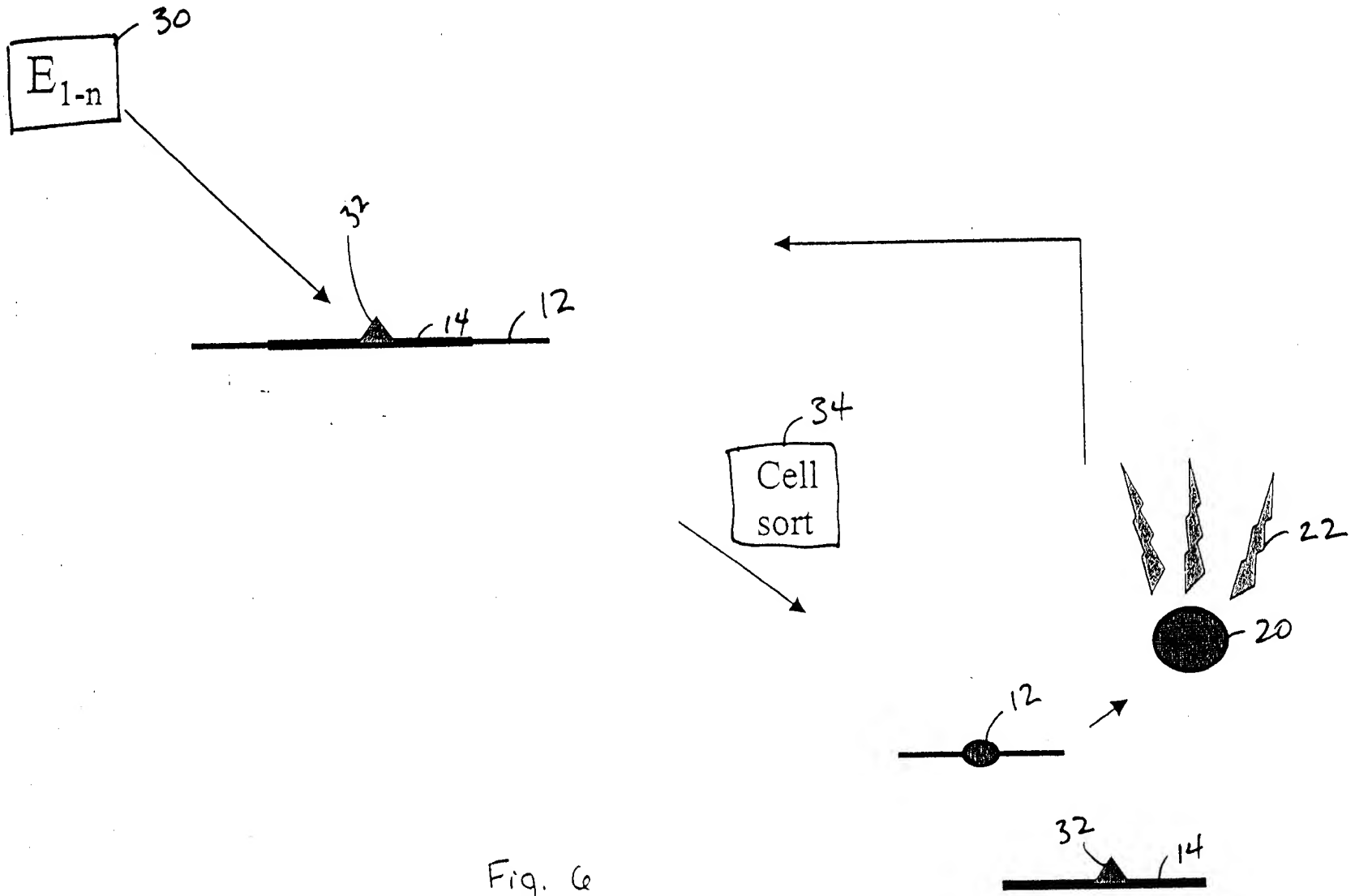


Fig. 6

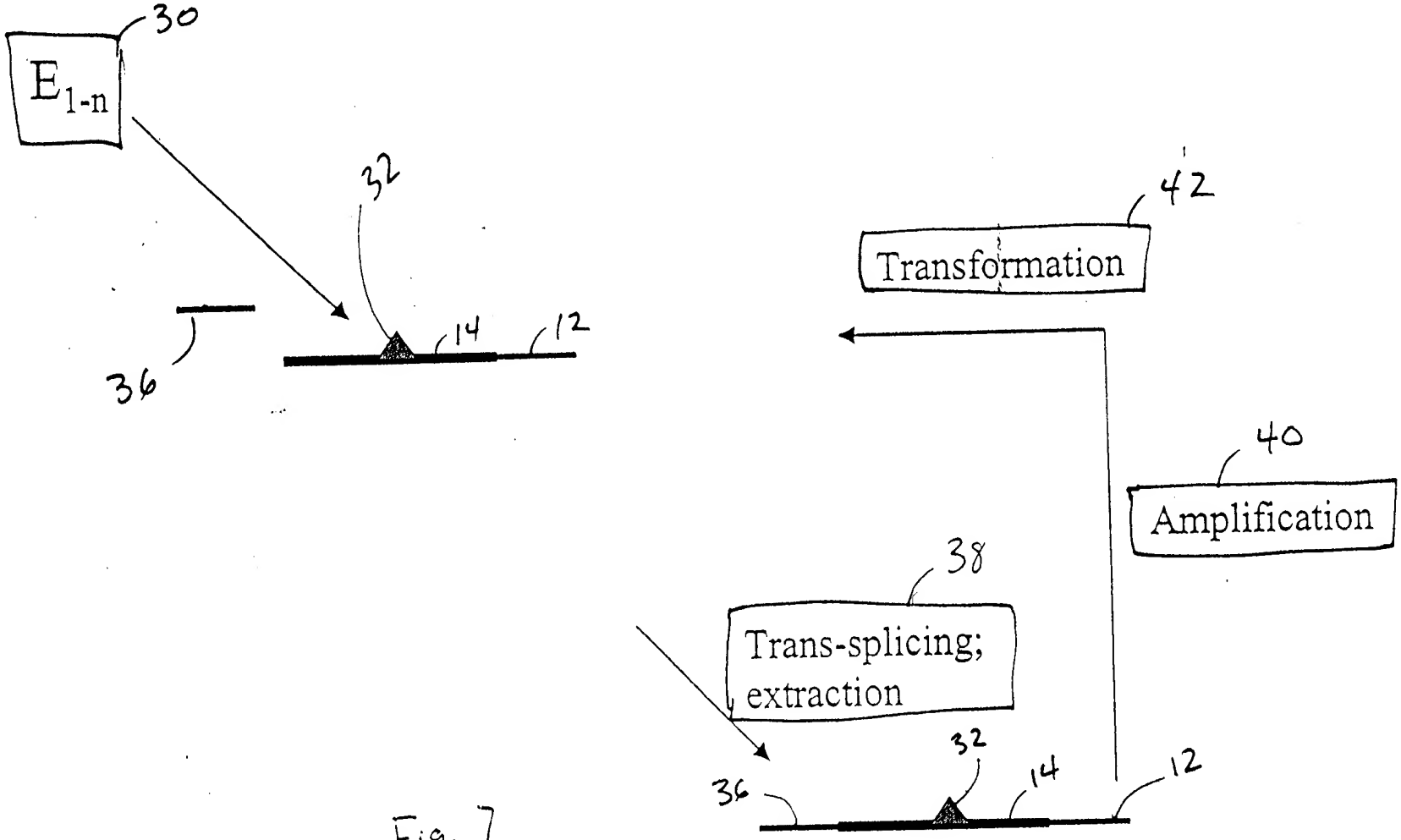

30
$E_{1\text{-}n}$
32
36
14
12
42
Transformation
40
Amplification
38
Trans-splicing;
extraction
32
36
14
12

Fig. 7

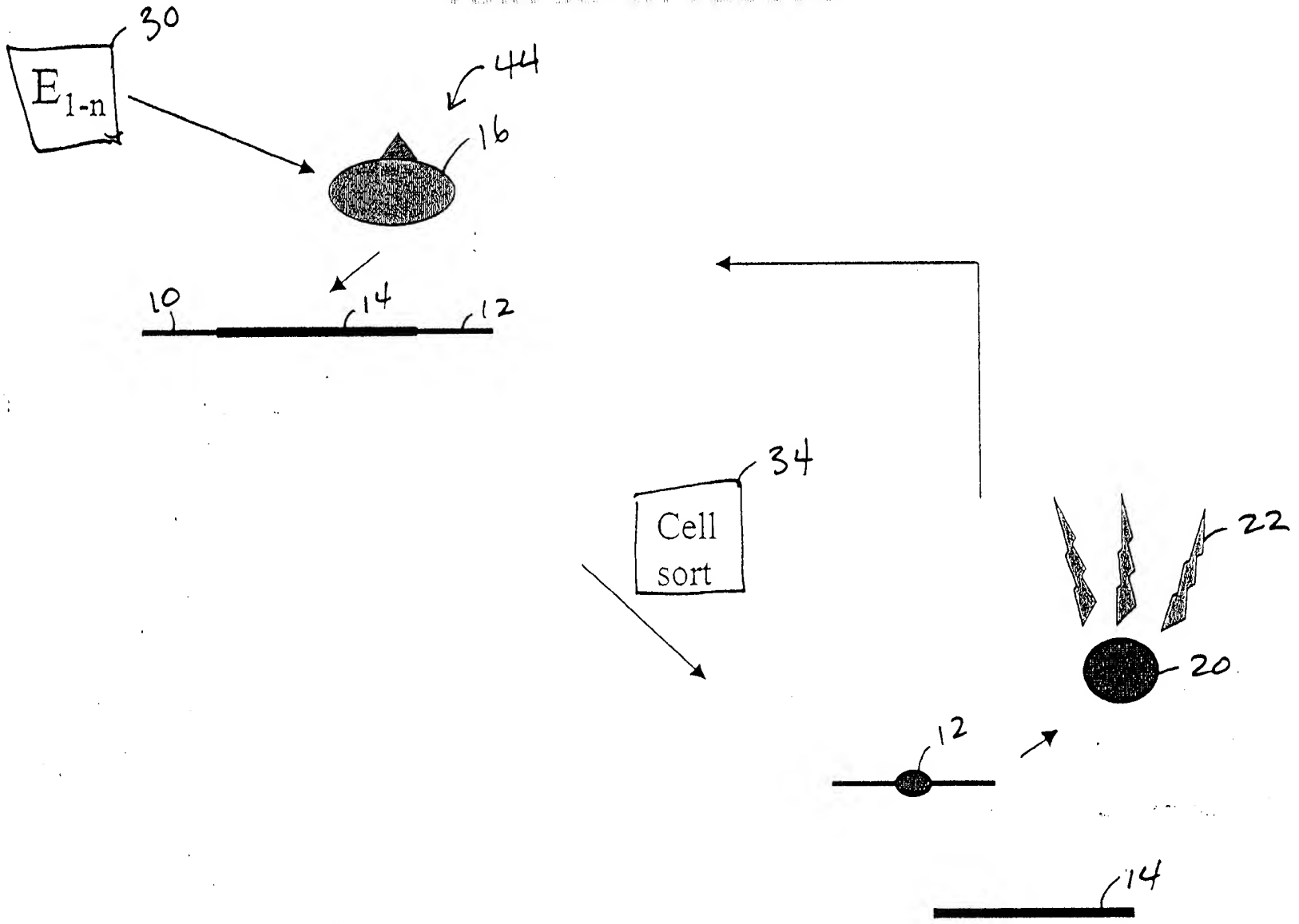


Fig. 8

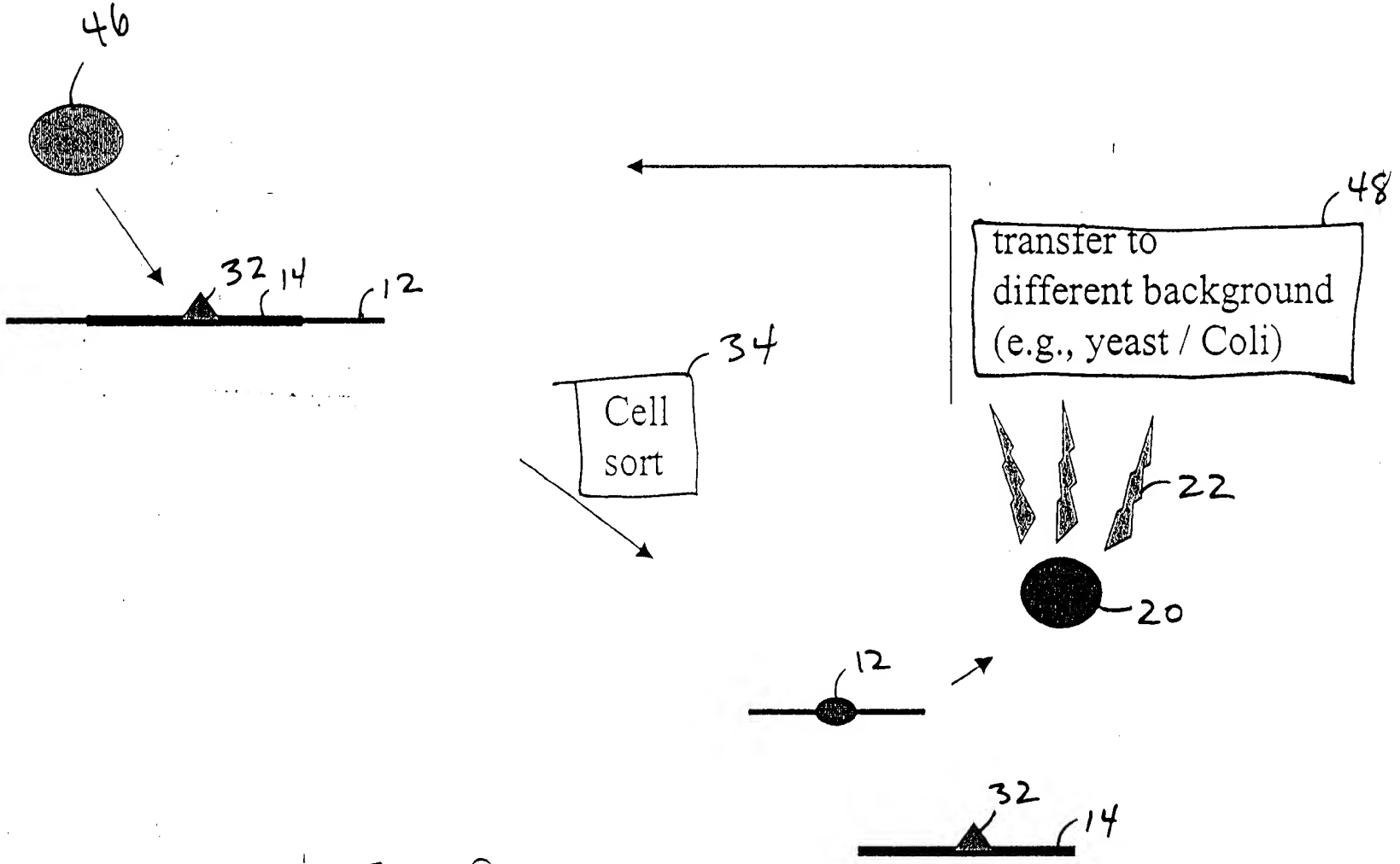

46
32
14
12
34
Cell sort
48
transfer to different background (e.g., yeast / Coli)
22
20
12
32
14

Fig. 9

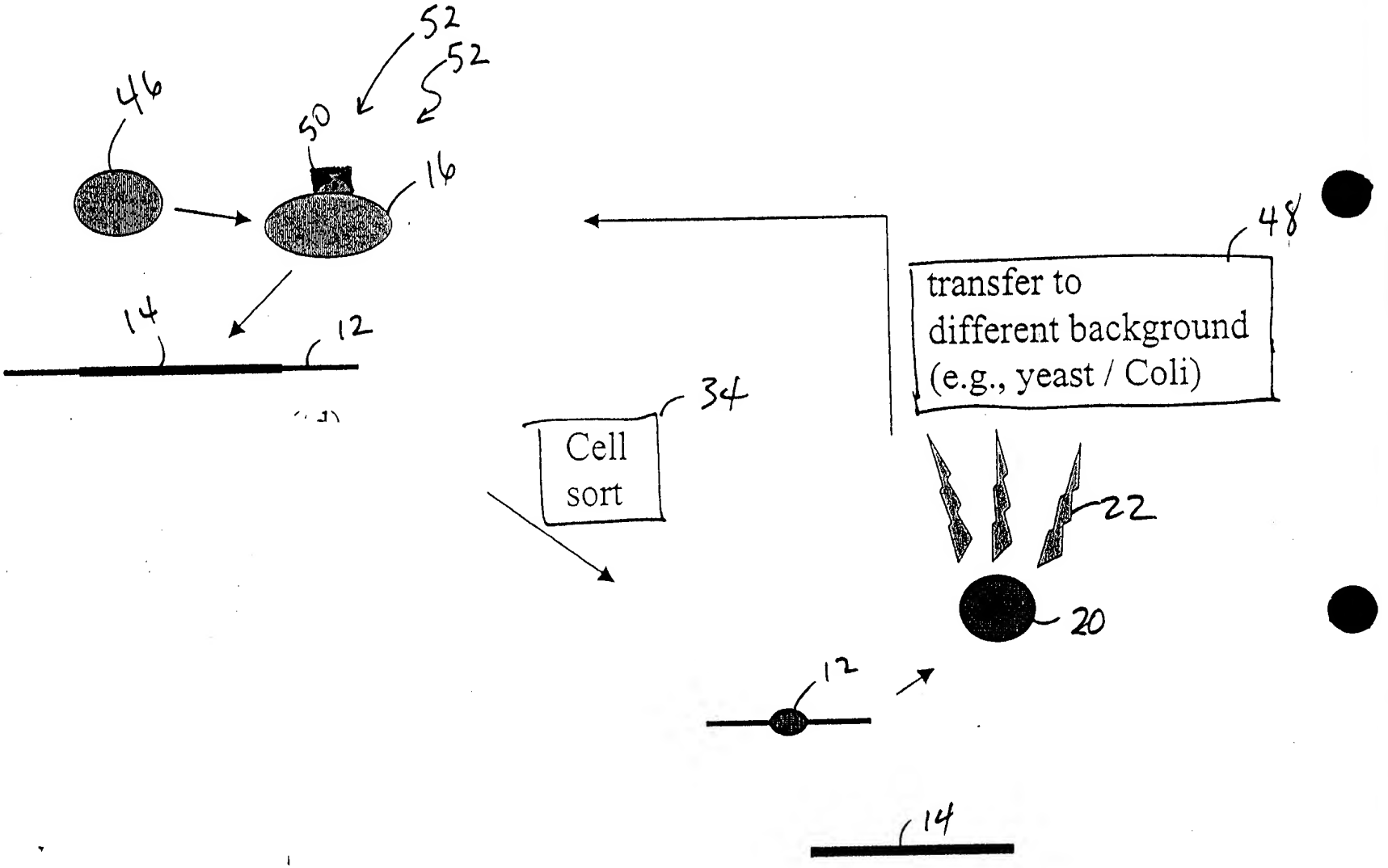

52
52
46
50
16
14
12
48
transfer to
different background
(e.g., yeast / Coli)
34
Cell
sort
22
20
12
14

Fig. 10

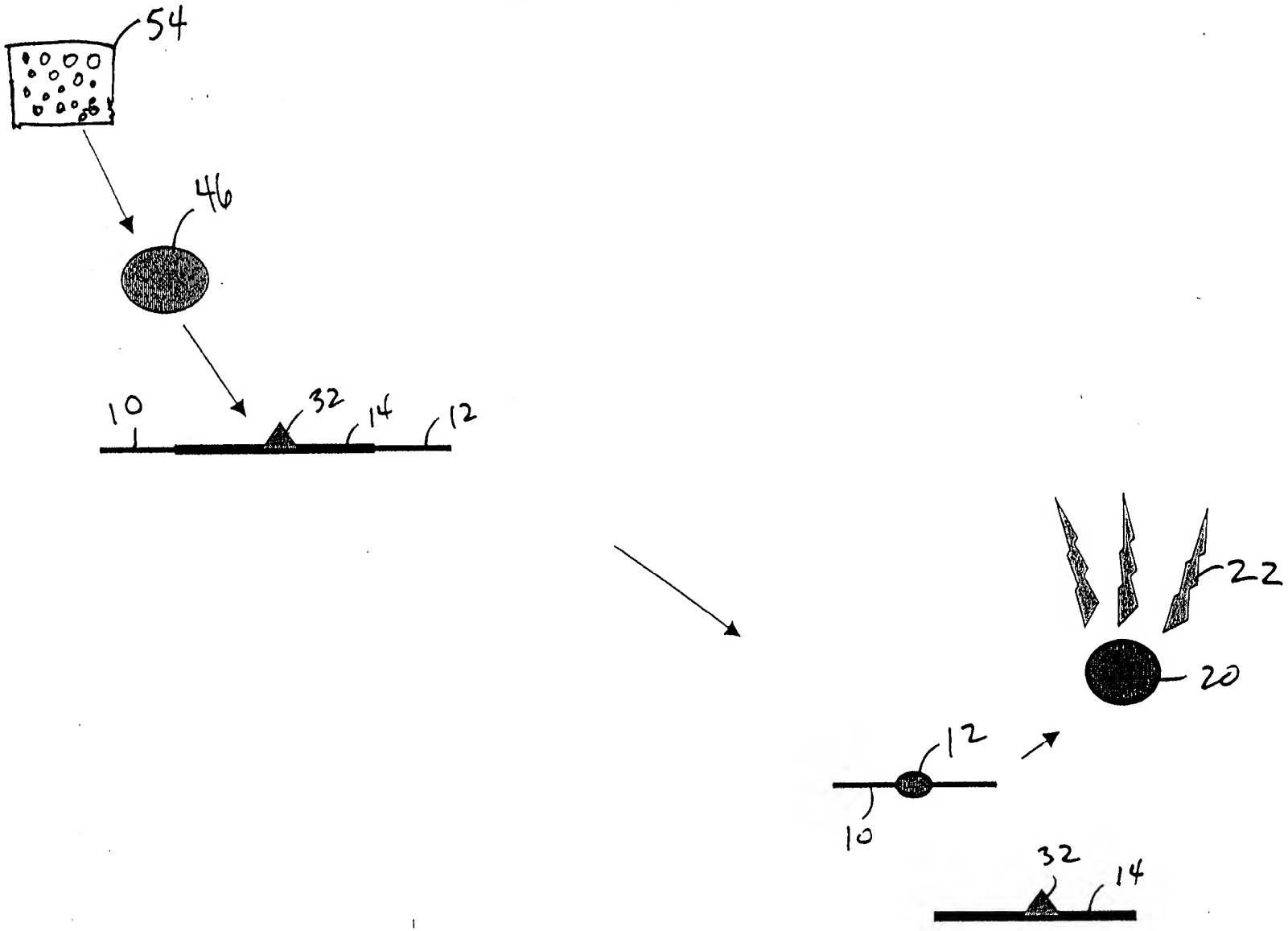


Fig. 11

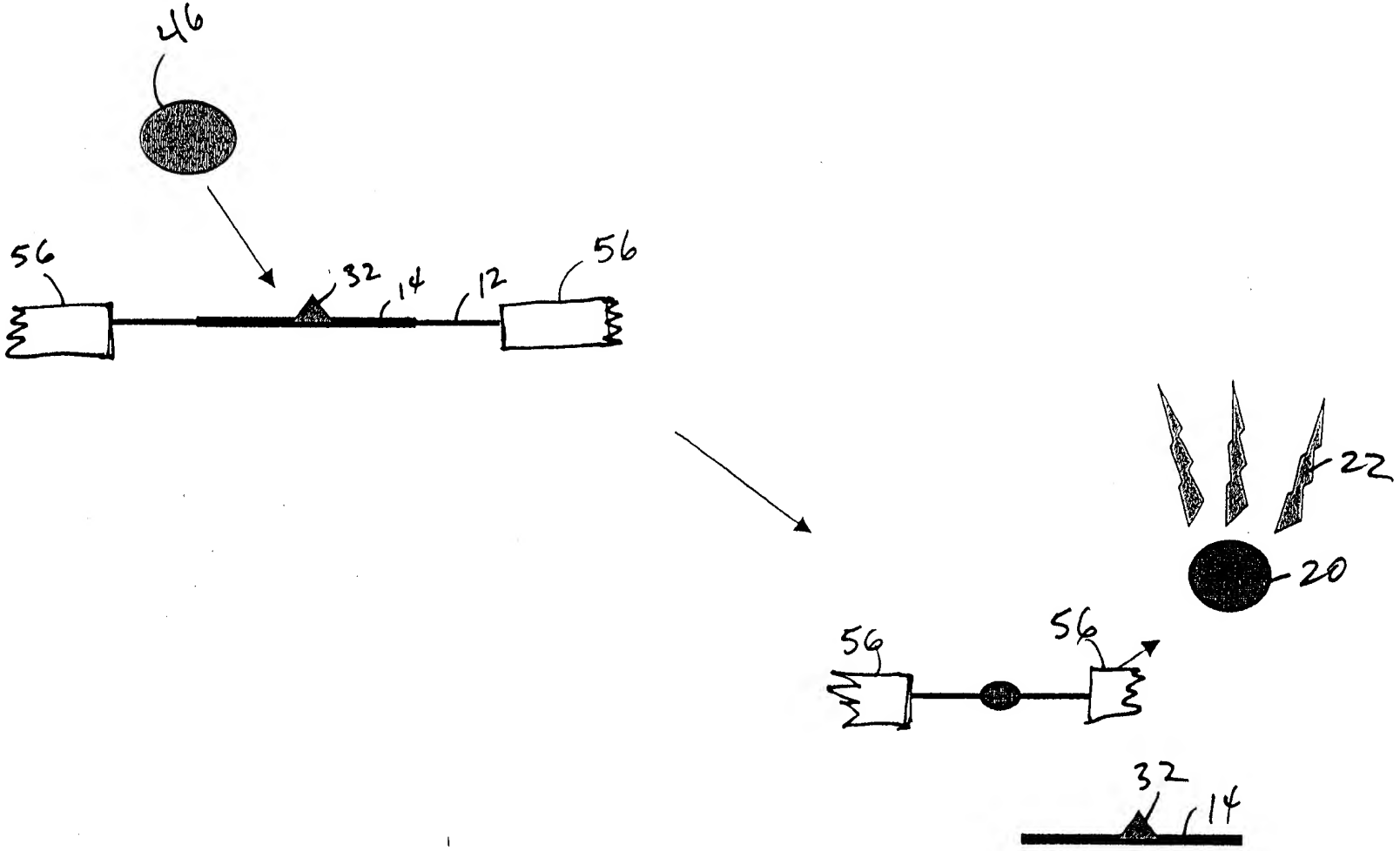


Fig. 12

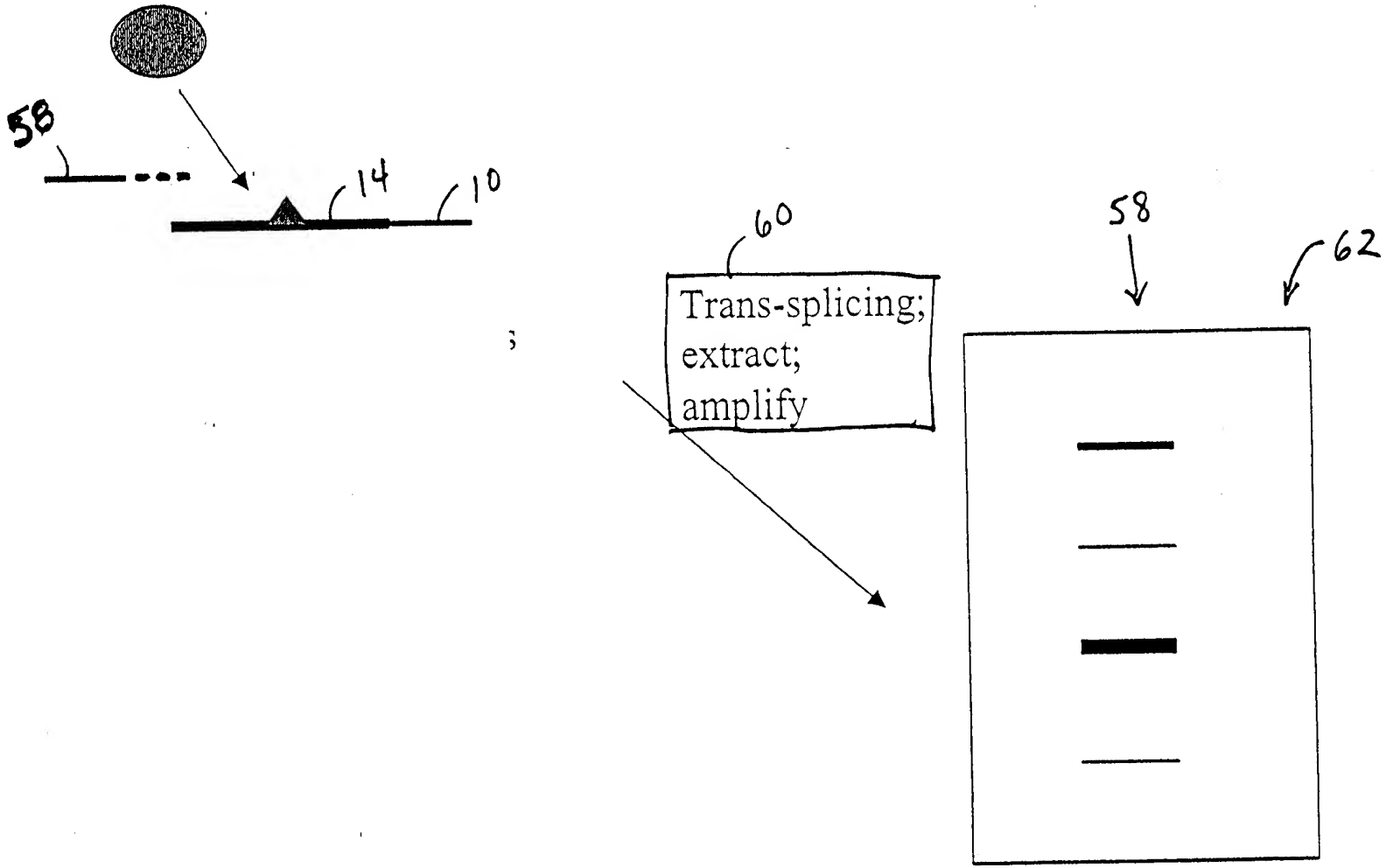


Fig. 13

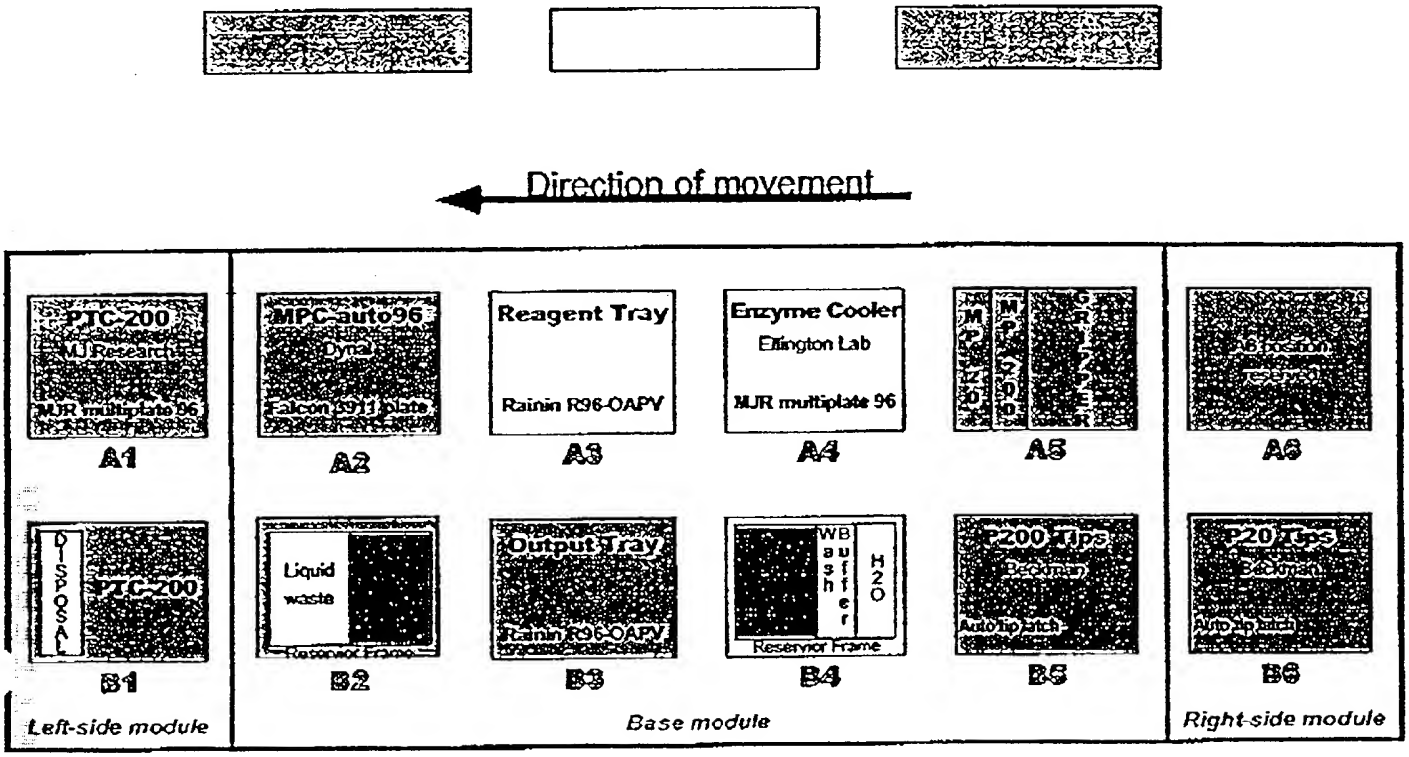

Direction of movement
PTC-200
MJ Research
MJR multiplate 96
A1
MPC-auto96
Dynal
Falcon 3911 plate
A2
Reagent Tray
Rainin R96-OAPV
A3
Enzyme Cooler
Ellington Lab
MJR multiplate 96
A4
A5
A6
DISPOSAL
PTC-200
B1
Liquid waste
Reservoir Frame
B2
Output Tray
Rainin R96-OAPV
B3
WB wash buffer
H2O
Reservoir Frame
B4
P200 Tips
Beckman
Auto tip pitch
B5
P20 Tips
Beckman
Auto tip pitch
B6
Left-side module
Base module
Right-side module

Fig. 14

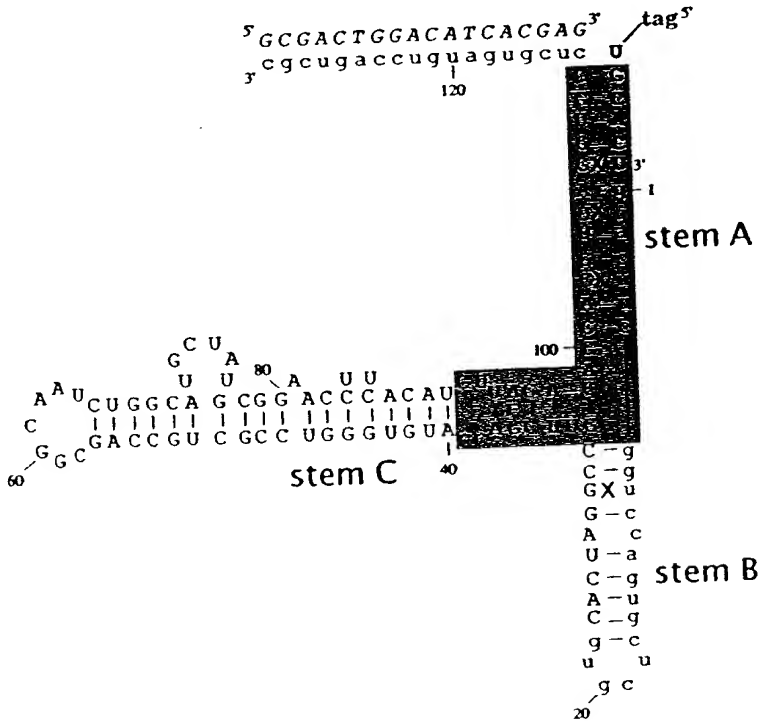


Fig. 15a

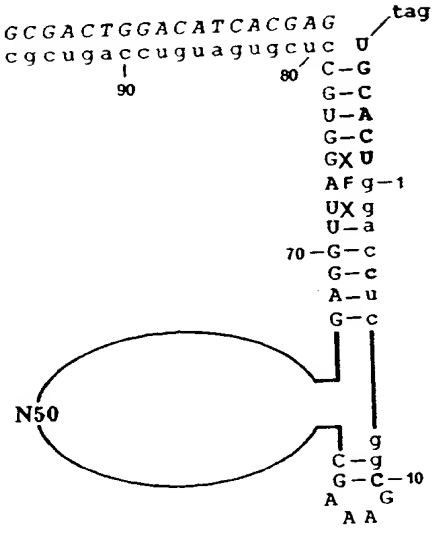


Fig. 15b

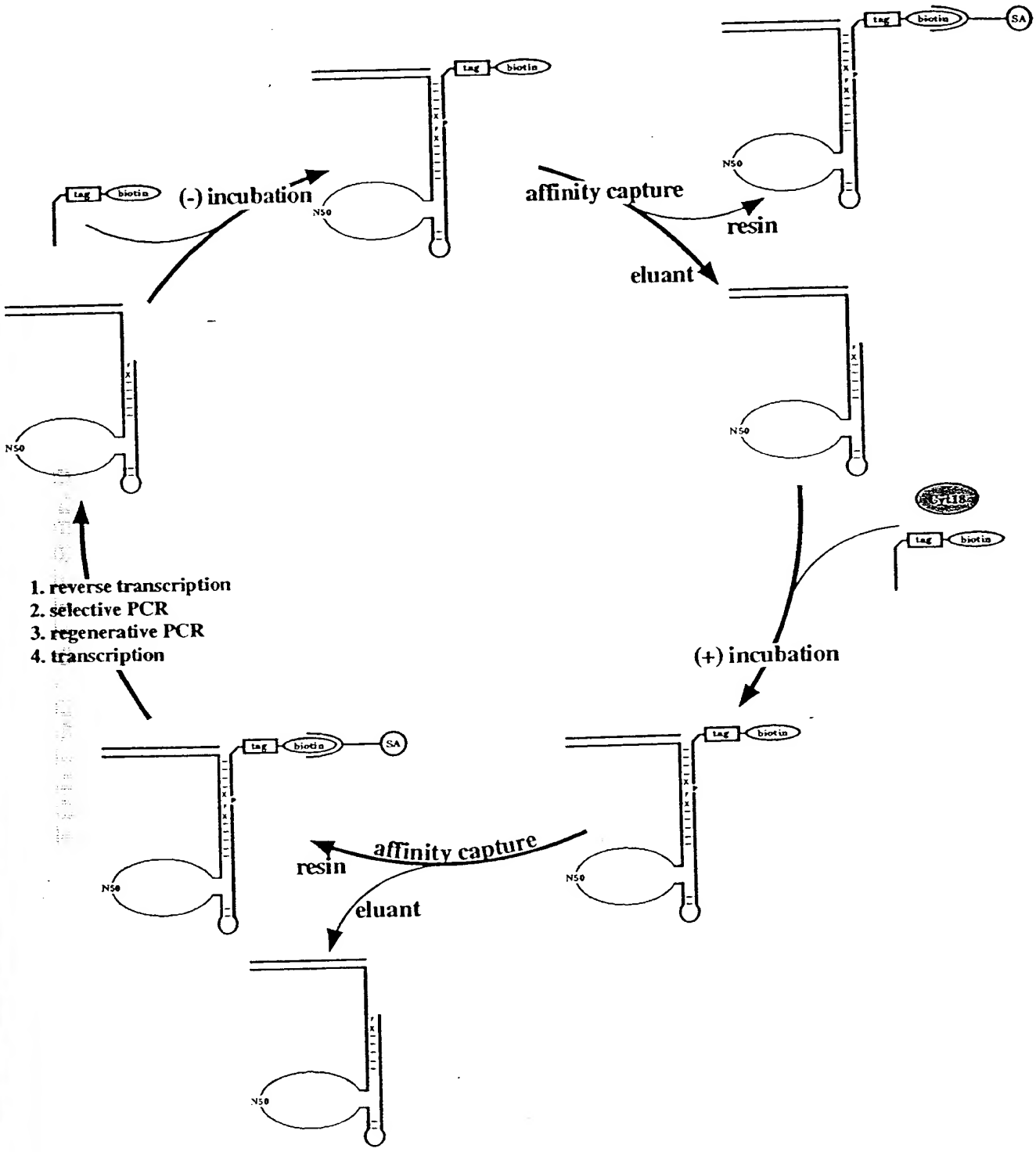

tag
biotin
SA
N50
(-) incubation
affinity capture
resin
eluant
1. reverse transcription
2. selective PCR
3. regenerative PCR
4. transcription
(+) incubation
affinity capture
resin
eluant

Fig. 15 c

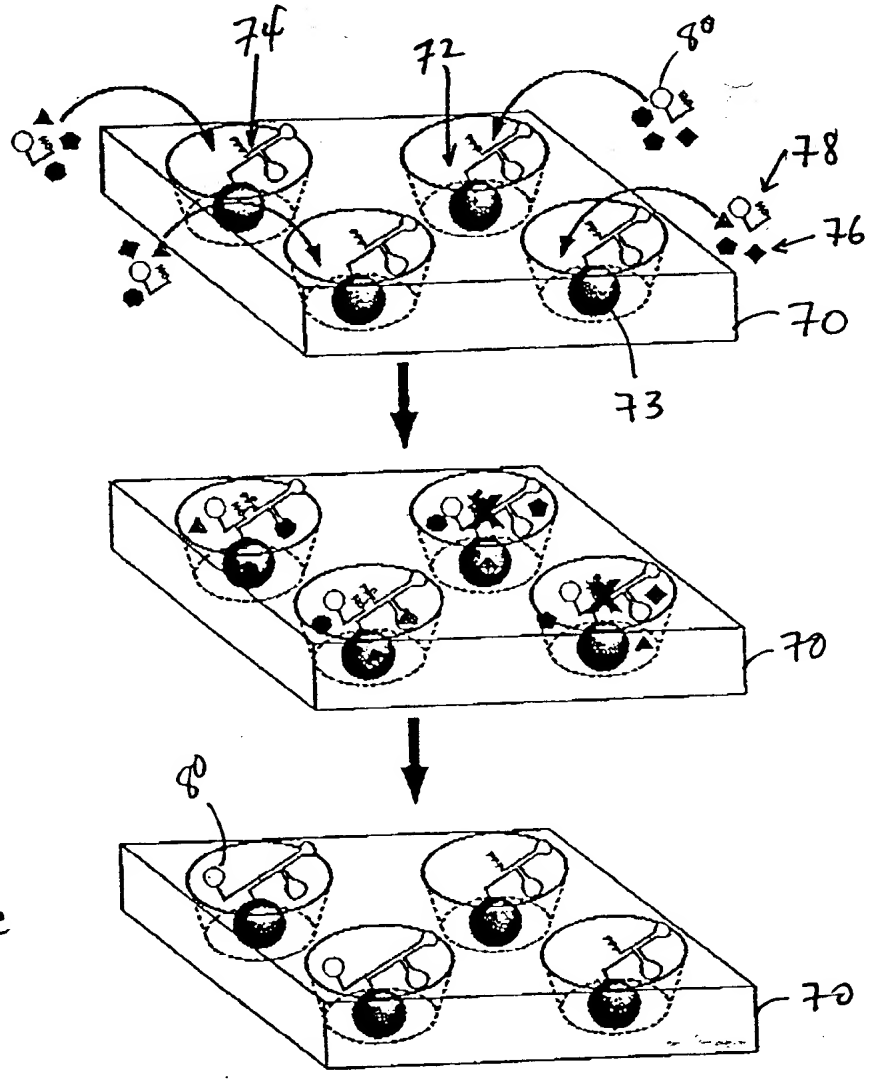


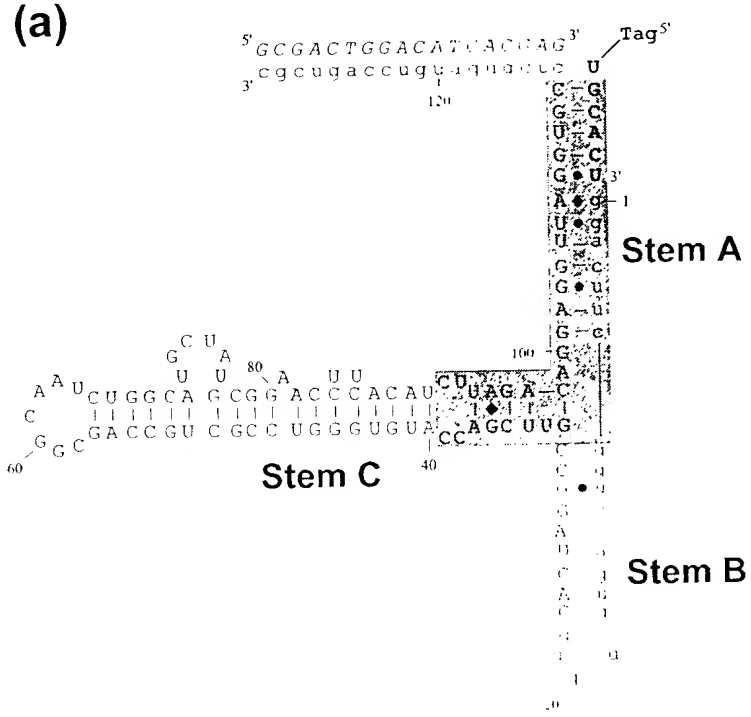
Fig 16a

Fig 16b

Fig 16c

Figure 17

(a)



(b)

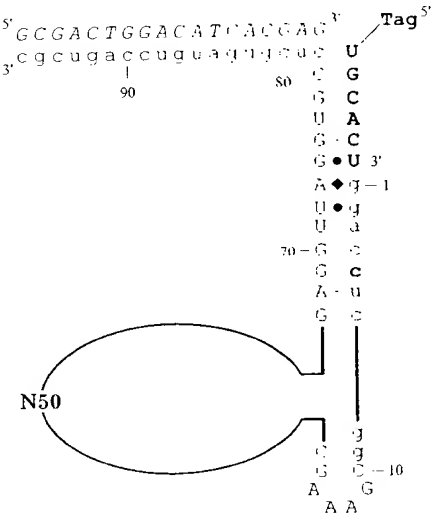


Figure 17

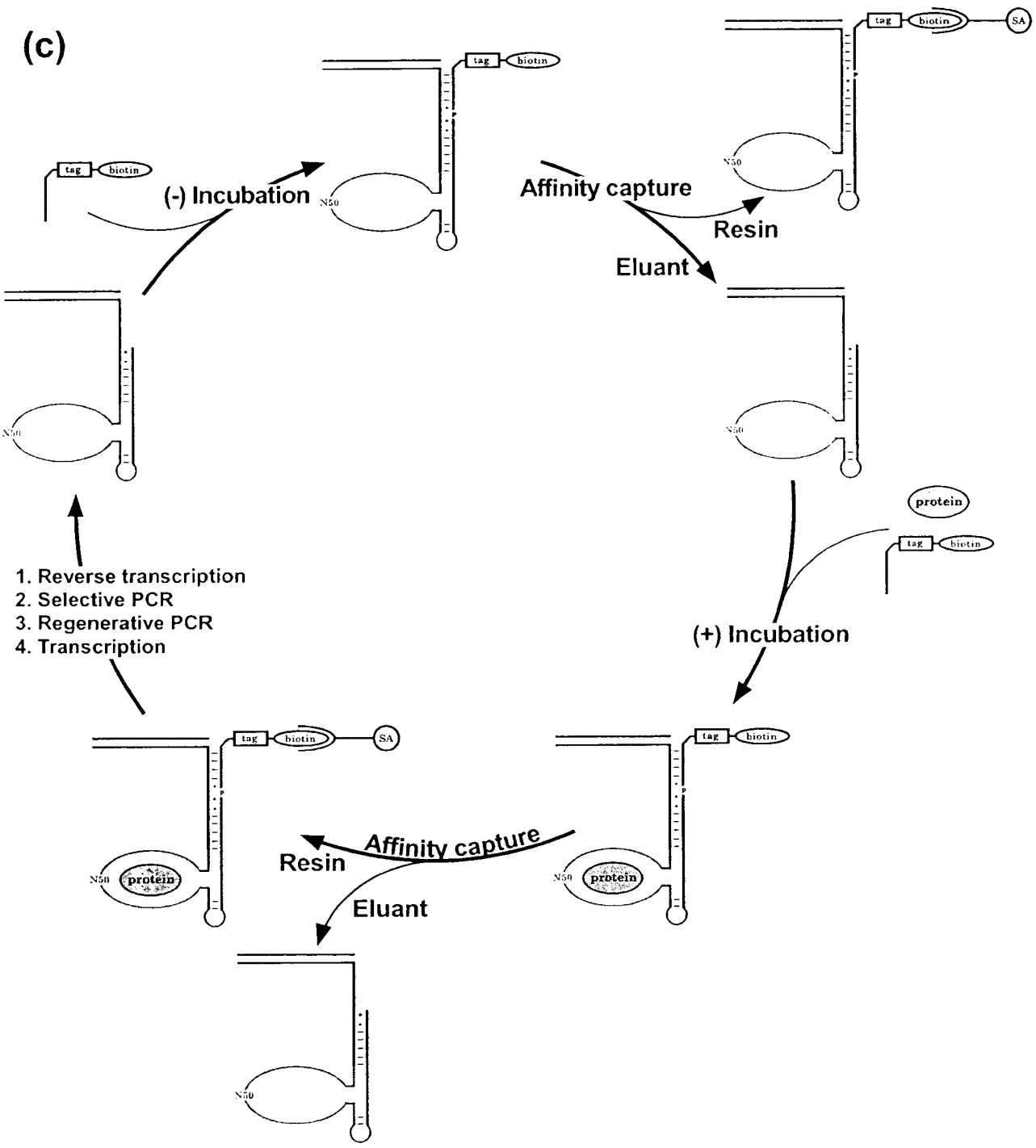

(c)
tag
biotin
SA
(-) Incubation
N50
Affinity capture
Resin
Eluant
1. Reverse transcription
2. Selective PCR
3. Regenerative PCR
4. Transcription
protein
(+) Incubation
Affinity capture
Resin
Eluant

Figure 18

(a)

Round	(-) Incubation Substrate	(-) Incubation (-) Cyt18	(+) Incubation Substrate	(+) Incubation (+) Cyt18	Activation
1			2X	14 h	
2	2X	20 h	2X	14 h	
3	2X	20 h	2X	8 h	0.9
4	2X	20 h	2X	1 h	1.0
5	4X	44 h	2X	1 h	26
6	4X	45 h	2X	15 min	1800
7	4X	91 h	2X	5 min	61000
8			1X	30 s	66000
9	4X	20 h	1X	30 s	76000

(b)

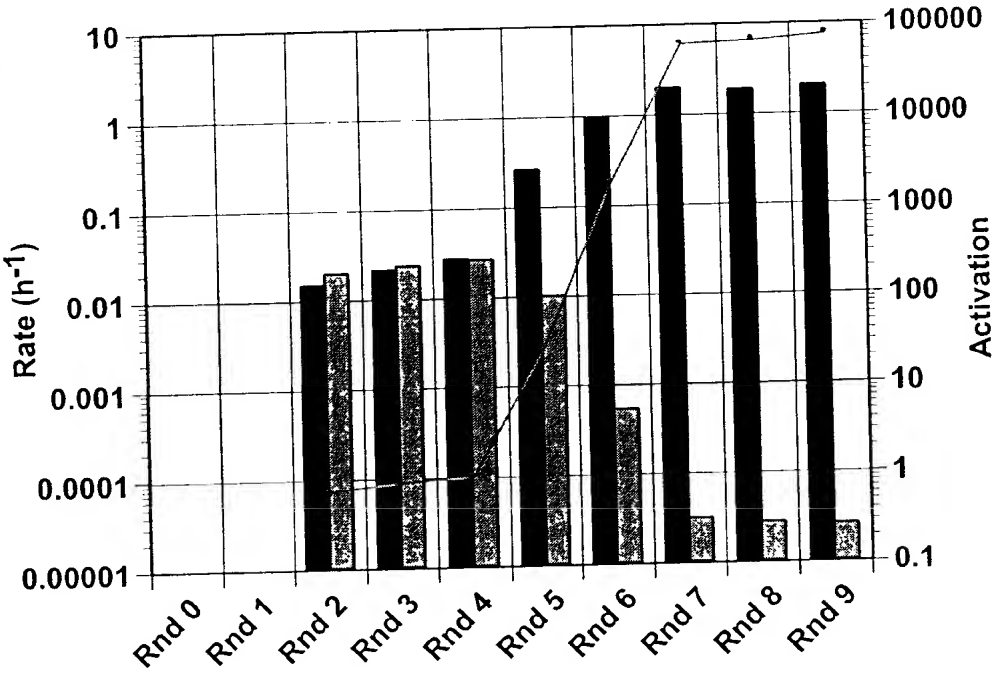


Figure 18

(c)

Round	(-) Incubation Substrate	(-) Lys	(+) Incubation Substrate	(+) Lys	Activation
1			2X	16 h	
2	2X	22 h	2X	14 h	1.0
3	2X	20 h	2X	8 h	1.0
4	2X	18 h	2X	1 h	1.0
5	4X	44 h	2X	1 h	1.0
6	4X	44 h	2X	15 min	1.7
7	4X	90 h	2X	15 min	5.0
8	4X	93 h	2X	5 min	270
9	4X	92 h	2X	1 min	630
10			1X	30 s	780
11	4X	118 h	1X	30 s	820

(d)

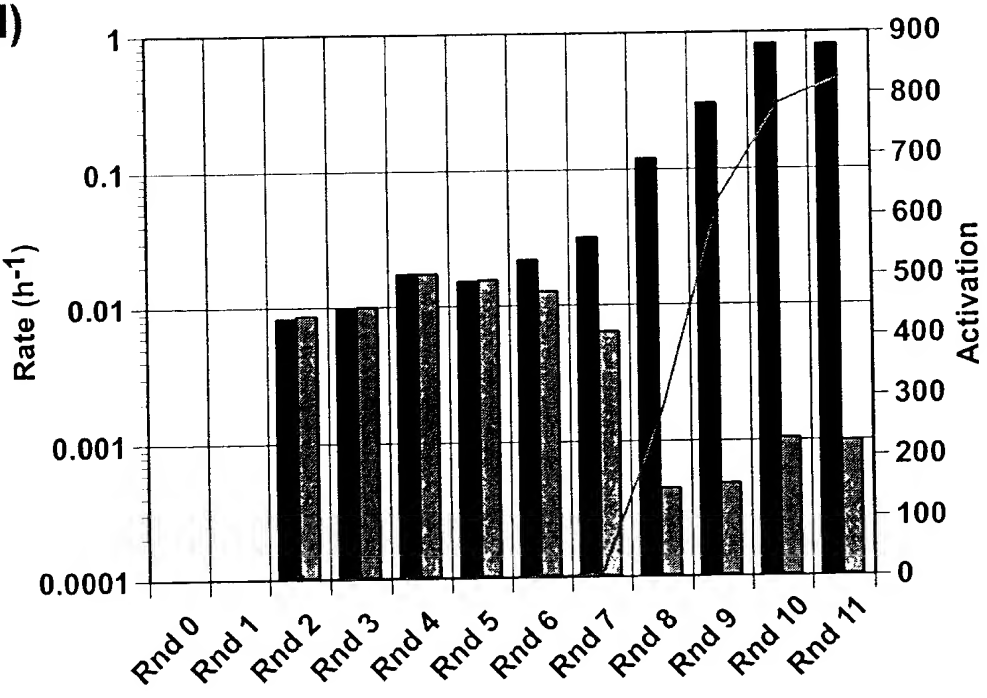


Figure 19

(a)

```
cyt7-2    (0.61)  CGGAAGCAAGGAGAGACGTCCTTGGAGGAGCAAGGG----------GTCTTACAGTCAGT
cyt7-6    (0.22)  CAGAGCATTAAGG----ACGGGTGACTCTTTAGTTAGGCTCCCGTTAGTCTTAGG
cyt7-1    (0.08)  CAGAGCATGAAGCGGCCACGGGTGGGATGTTGCCCTTG-----GTCAGTCTTGGG
cyt9-2    (0.03)  AGGAACCCCCAGATTGTGTCGGGCTGTTATGCGTCGTTTATTGAGATTAC
cyt9-16   (0.03)  CAGTACGTTAATATCCCGGAGCTAGGTGCTTCTTGTGGACAGTTATGGG
cyt9-18   (0.03)  GCACACAGCACTATATTGCTTGGTCGGAGCGTTTCGTTTATTGAGTTTAC

lys11-2   (0.50)  TAACGTCTCATGGCTAAATTGCCATGT-TGCTACAAATGATATGACTAGA
lys11-3   (0.38)  TAACGAAGACTTTGGTGACCGGCTAGTCTTCTATTAATGAGATGACGAGA
lys11-28  (0.08)  TAACTCCCGCACTTAGGAACGGGTGCTGGA-TAAAAATGATATGACGAGA
lys11-6   (0.04)  TTTAAAACGAGAGAATTGGCAGTACCGTGCT-GGTTCCGAGATAACGAGA
```

(b)

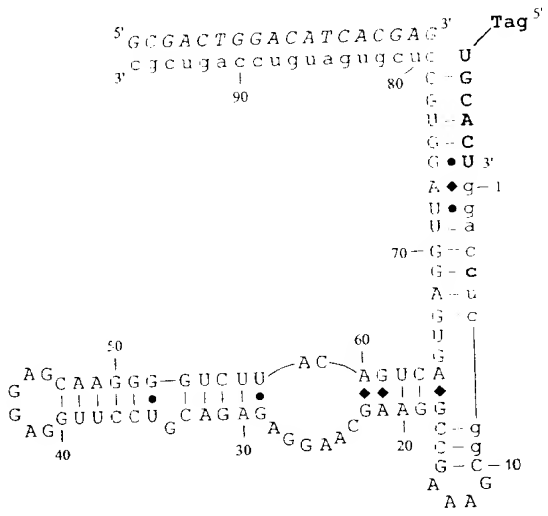


Figure 20

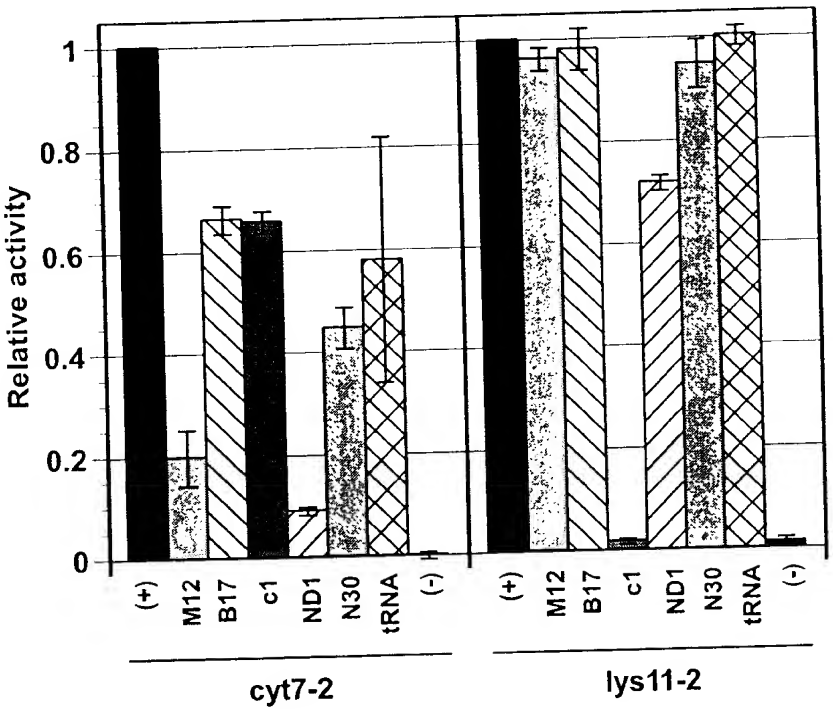

Relative activity
1
0.8
0.6
0.4
0.2
0
(+)
M12
B17
c1
ND1
N30
tRNA
(-)
cyt7-2
lys11-2

Figure 21

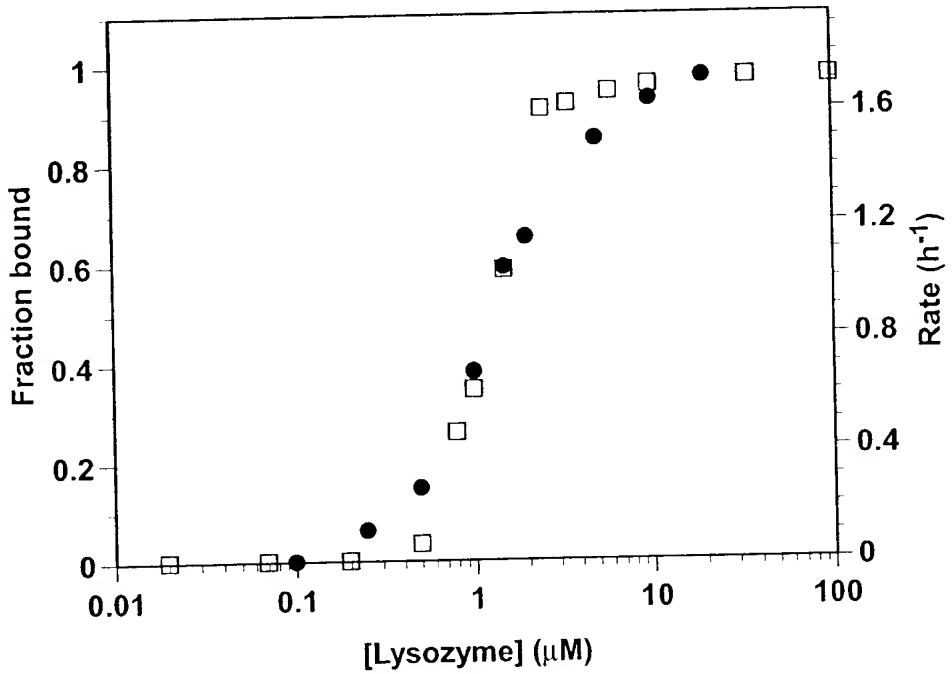

Fraction bound
Rate (h^{-1})
[Lysozyme] (μM)
0
0.2
0.4
0.6
0.8
1
0
0.4
0.8
1.2
1.6
0.01
0.1
1
10
100

Figure 22

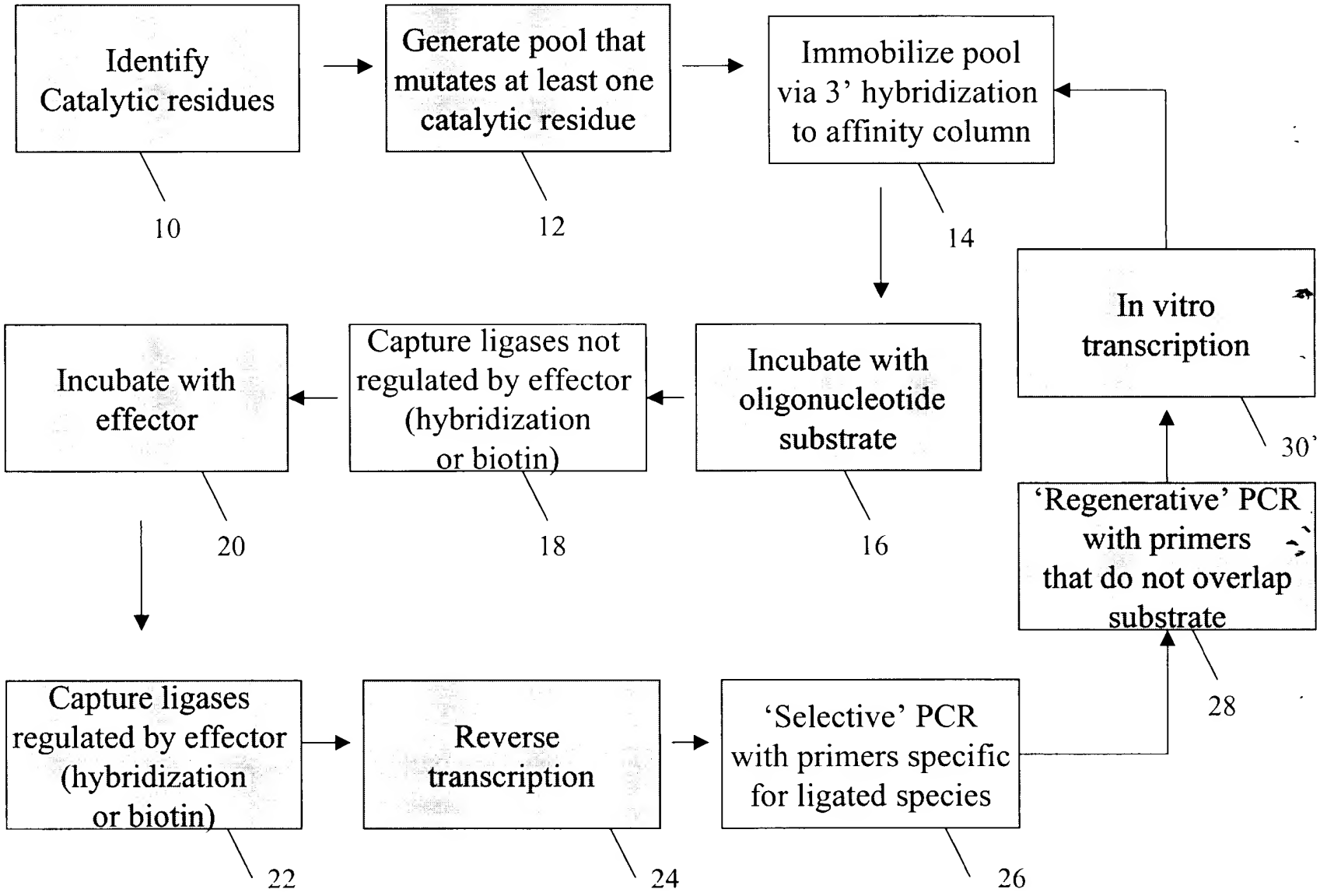

Identify Catalytic residues
10
Generate pool that mutates at least one catalytic residue
12
Immobilize pool via 3' hybridization to affinity column
14
In vitro transcription
30'
'Regenerative' PCR with primers that do not overlap substrate
28
'Selective' PCR with primers specific for ligated species
26
Reverse transcription
24
Capture ligases regulated by effector (hybridization or biotin)
22
Incubate with effector
20
Capture ligases not regulated by effector (hybridization or biotin)
18
Incubate with oligonucleotide substrate
16

FIGURE 23

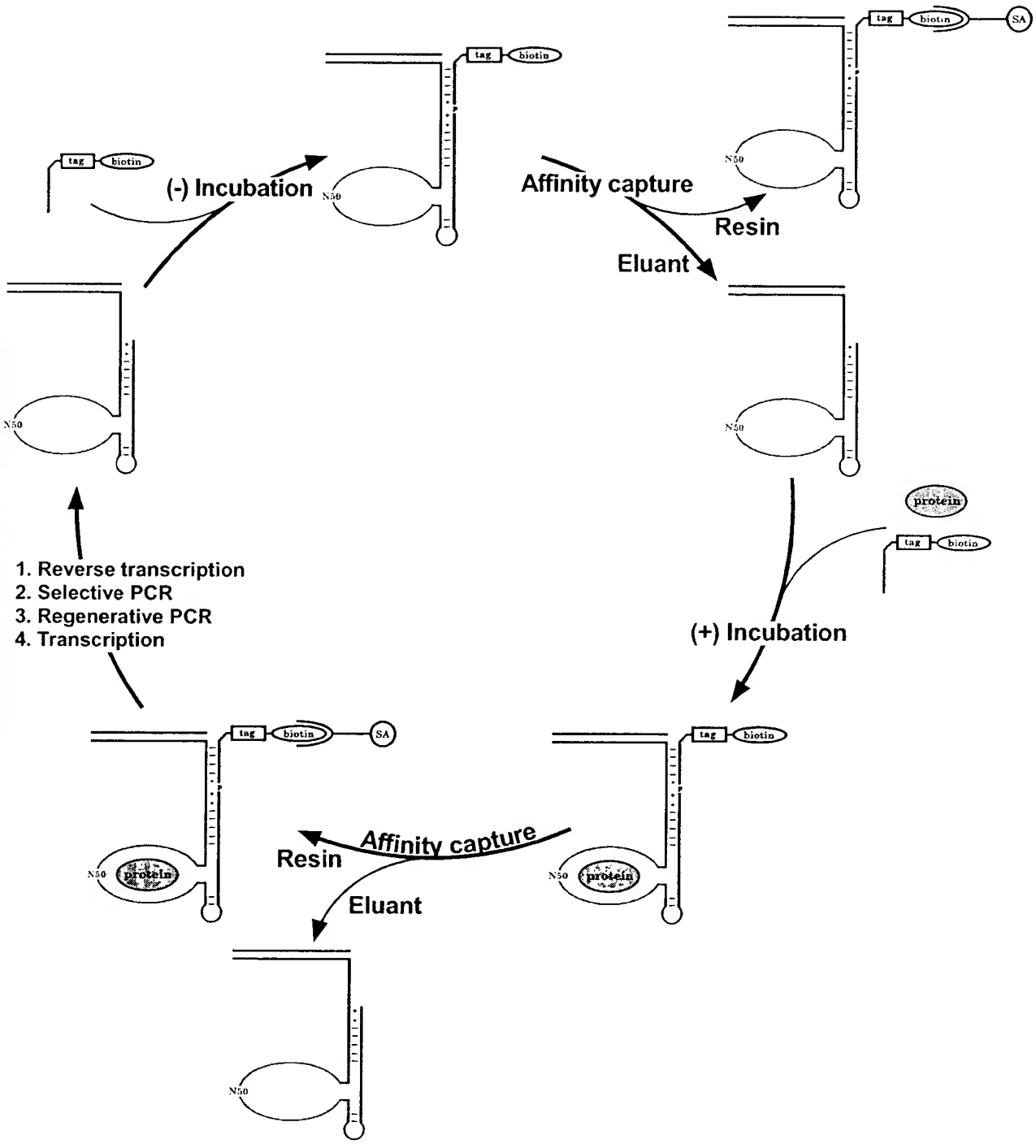
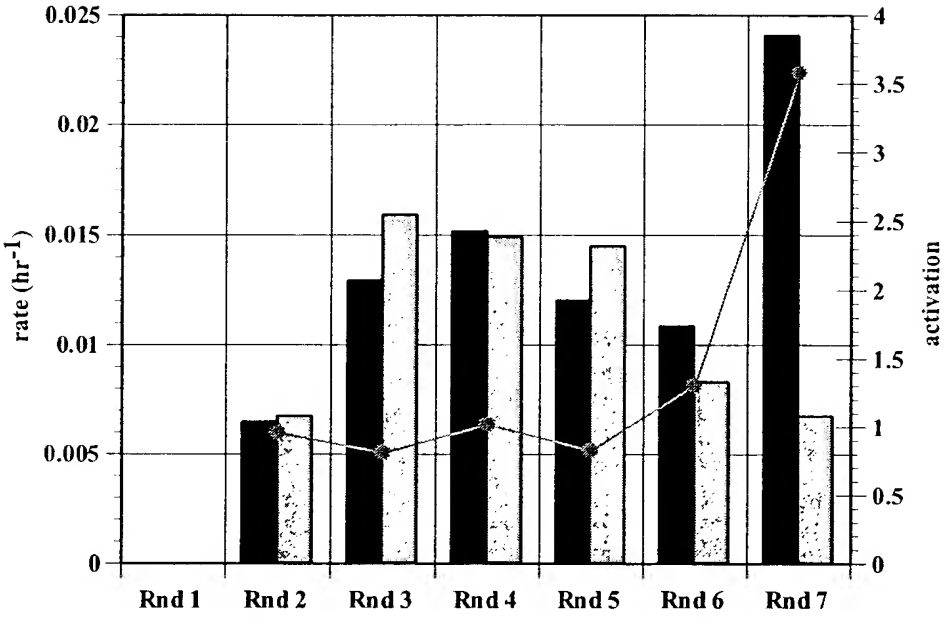

tag
biotin
SA
(-) Incubation
N50
Affinity capture
Resin
Eluant
1. Reverse transcription
2. Selective PCR
3. Regenerative PCR
4. Transcription
protein
(+) Incubation
Affinity capture
Resin
Eluant

FIGURE 24


0.025
0.02
0.015
0.01
0.005
0
rate (hr-1)
4
3.5
3
2.5
2
1.5
1
0.5
0
activation
Rnd 1
Rnd 2
Rnd 3
Rnd 4
Rnd 5
Rnd 6
Rnd 7

a)

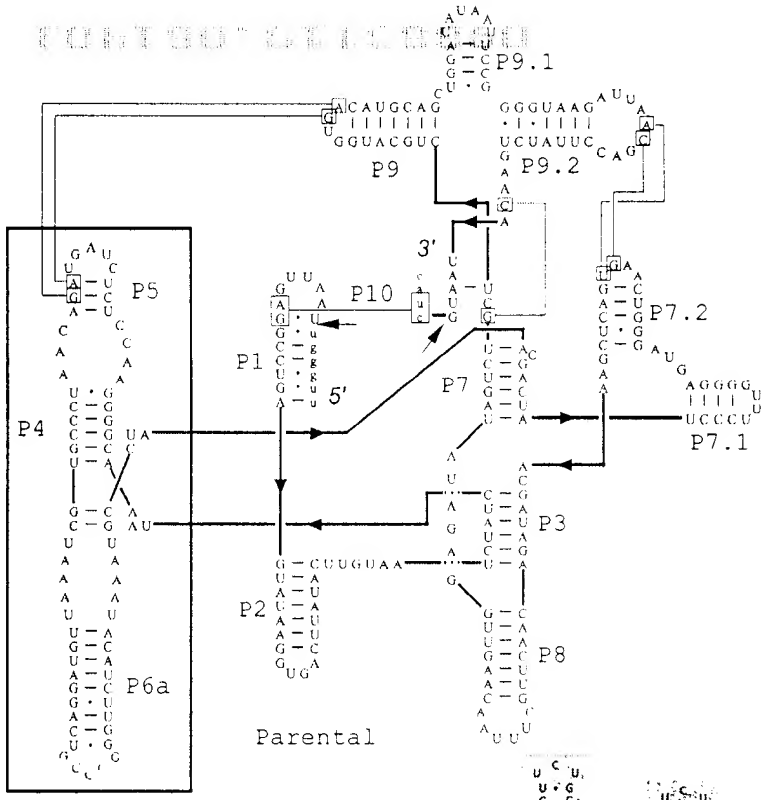
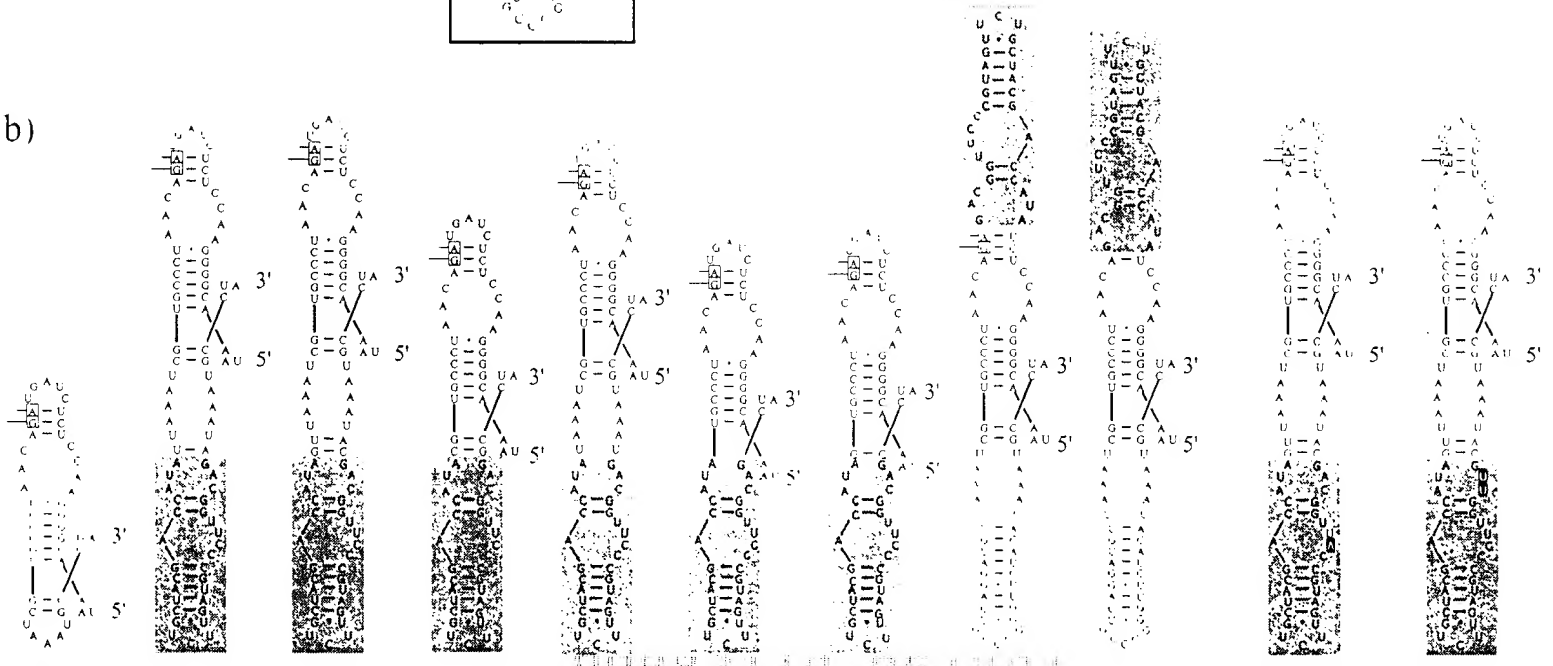
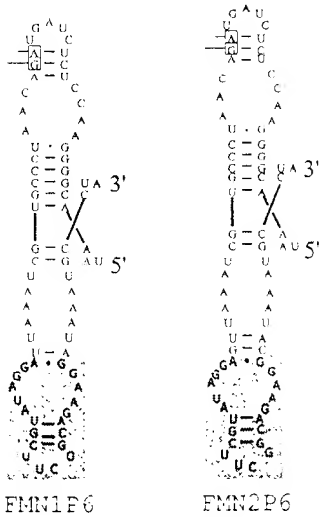

P9.1
P9
P9.2
P10
P5
P4
P1
P7
P7.2
P7.1
P3
P2
P8
P6a
5'
3'
Parental

FIGURE 25

b)


B11
Th1P6
Th2P6
Th3P6
Th4P6
Th5P6
Th6P6
Th1P5
Th2P5
3MeX3P6
Th2P6.D



FMN1P6 FMN2P6

FIGURE 26

FIGURE 27(a)

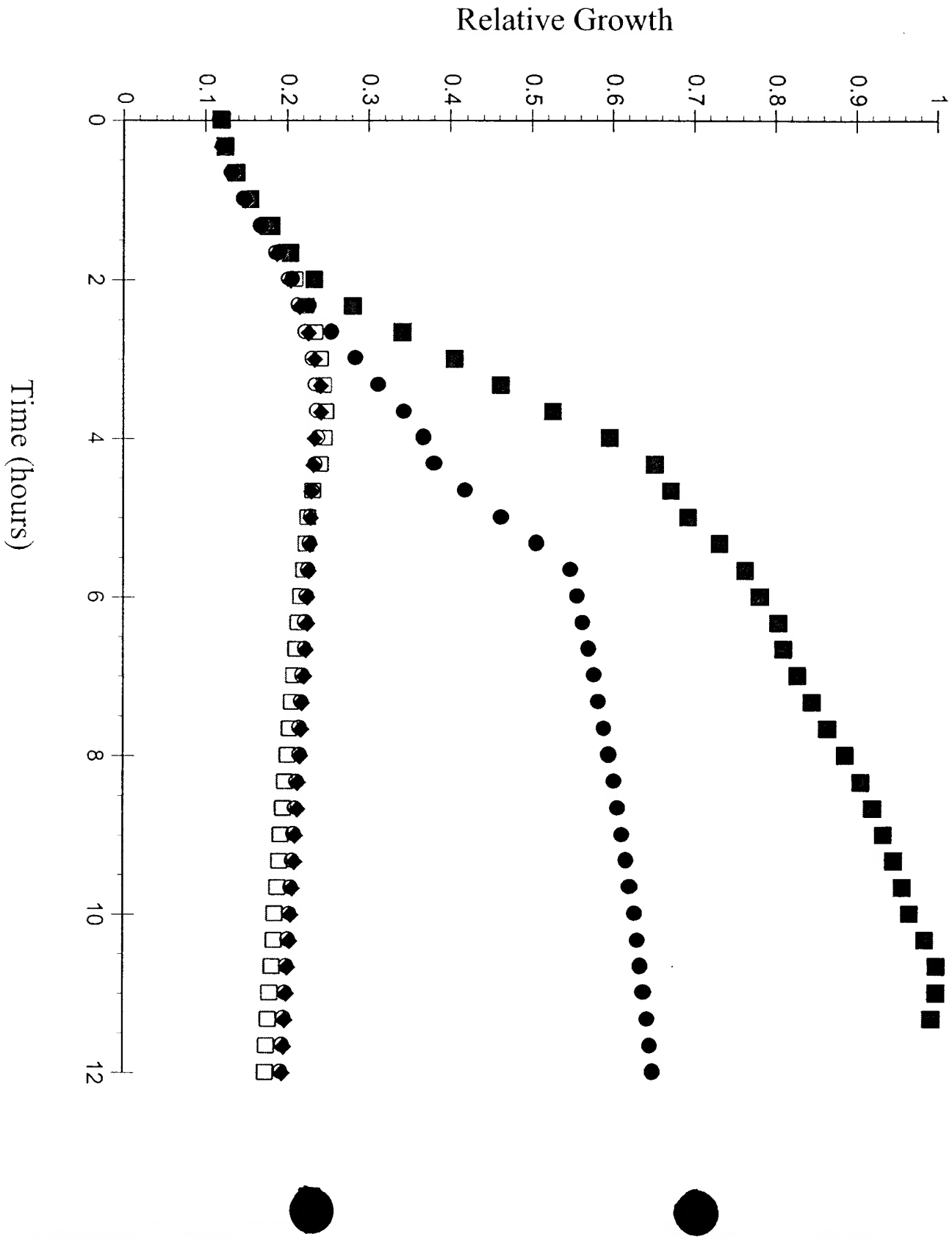

Relative Growth
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Time (hours)
0
2
4
6
8
10
12

FIGURE 27(b)

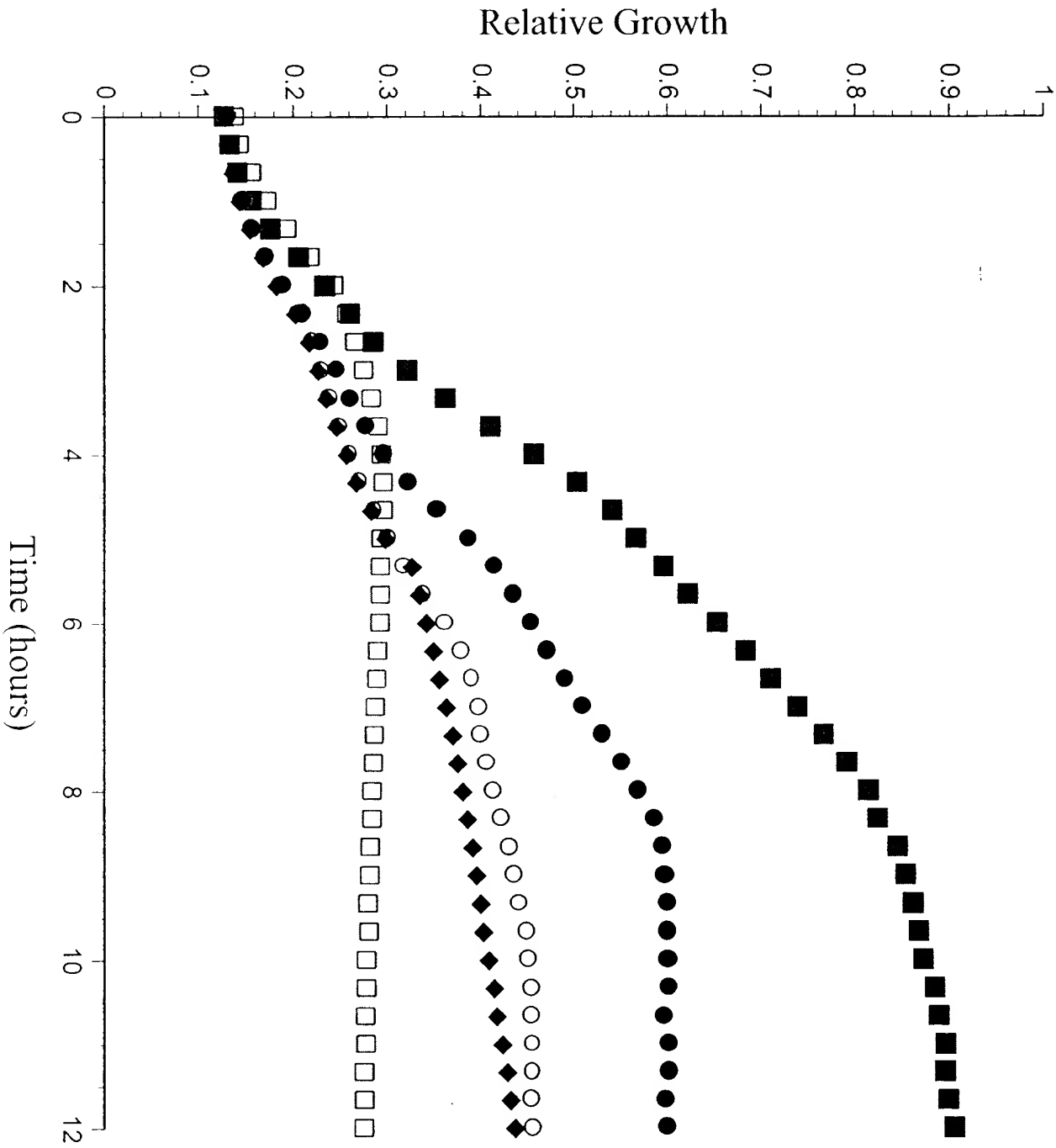

Relative Growth
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Time (hours)
0
2
4
6
8
10
12

FIGURE 27(c)

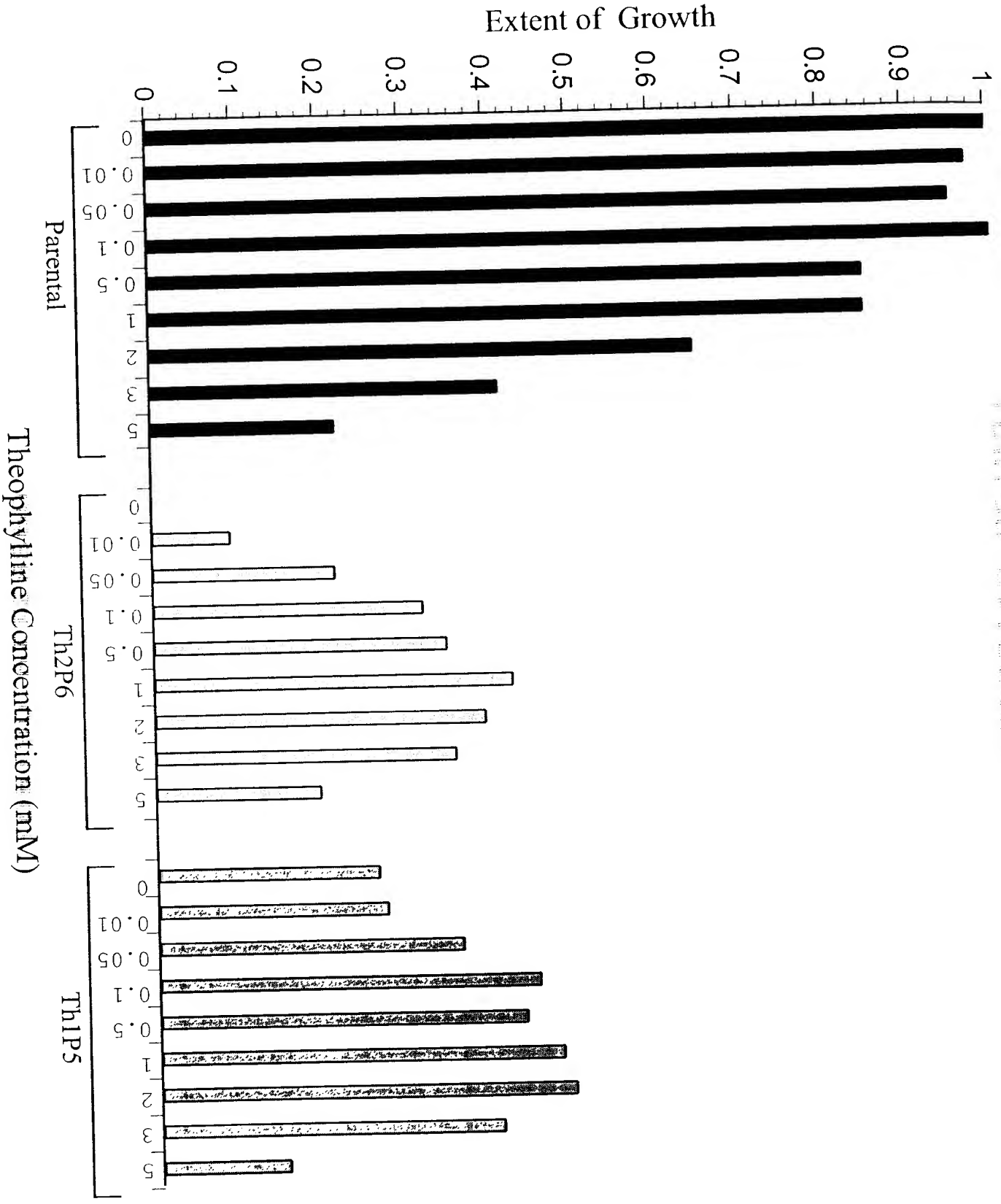

Extent of Growth
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Parental
Th2P6
Th1P5
Theophylline Concentration (mM)
0
0.01
0.05
0.1
0.5
1
2
3
5

FIGURE 28

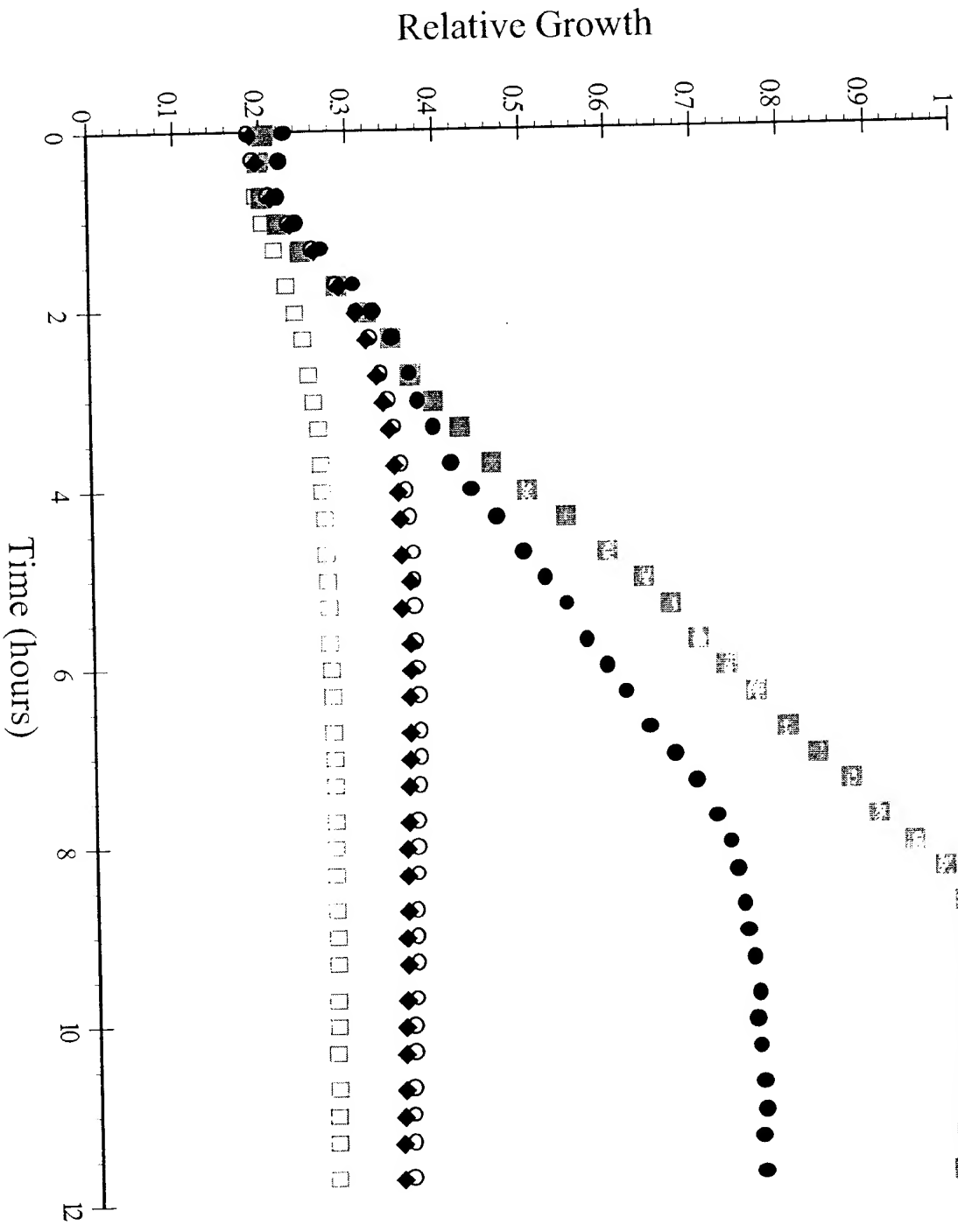

Relative Growth
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Time (hours)
0
2
4
6
8
10
12

FIGURE 29

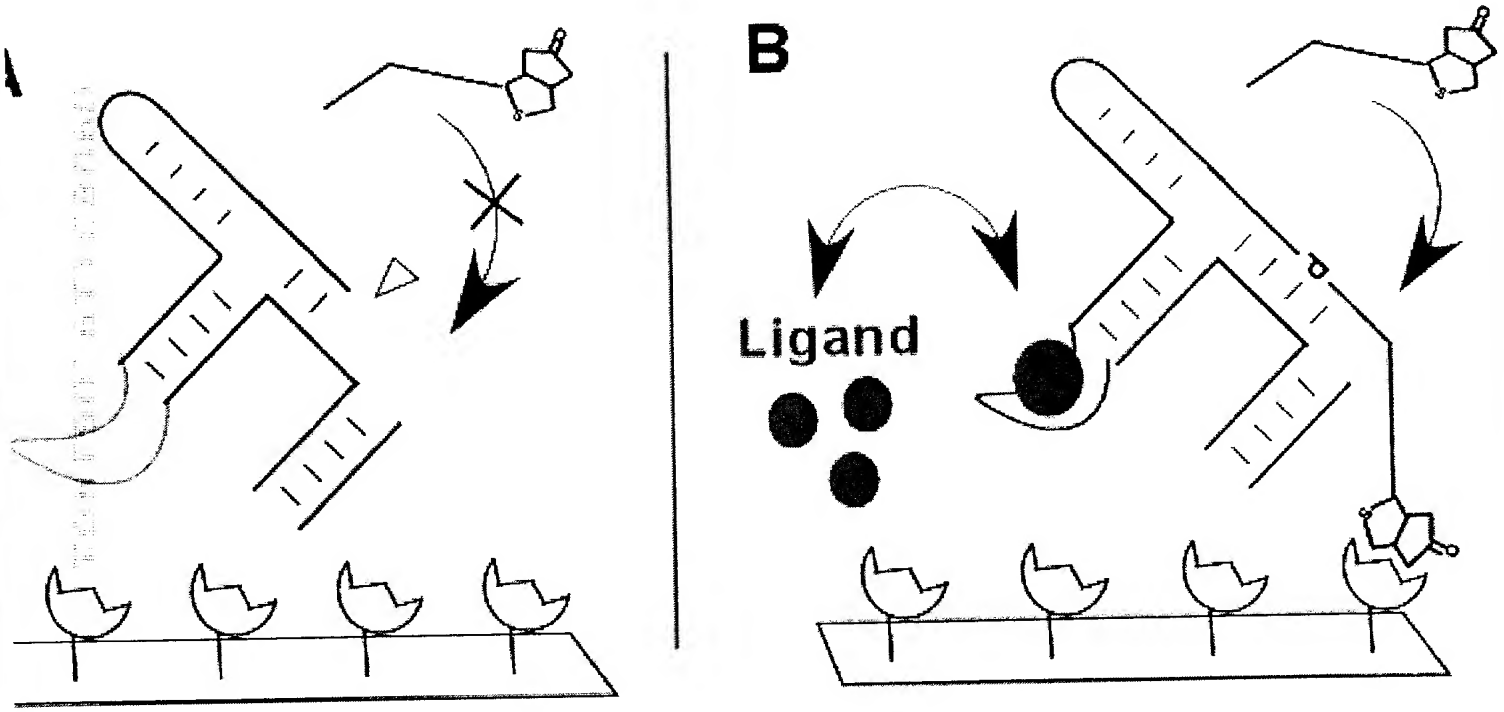


FIGURE 30

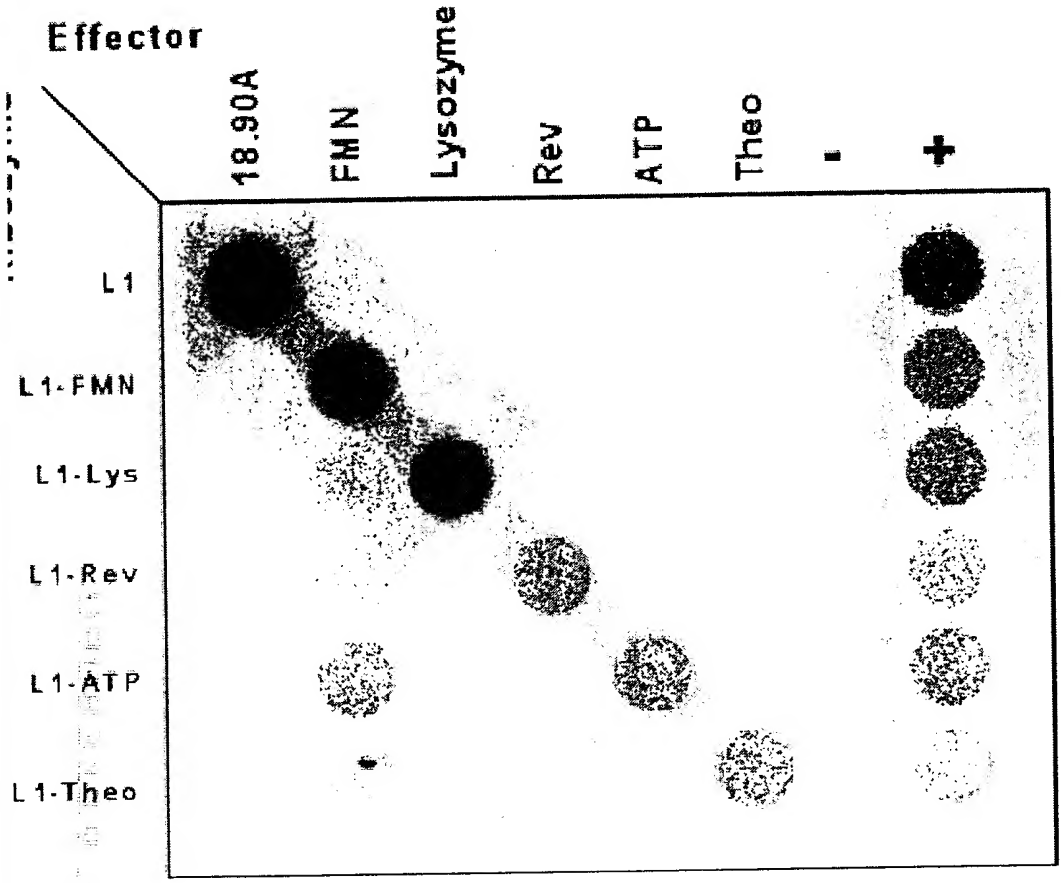
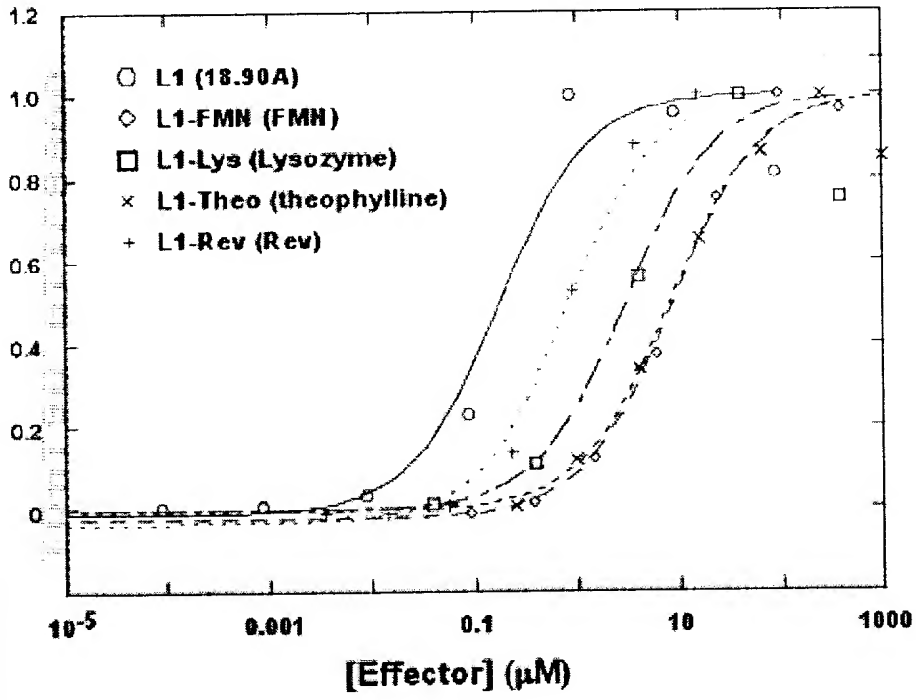

Effector
18.90A
FMN
Lysozyme
Rev
ATP
Theo
-
+
L1
L1-FMN
L1-Lys
L1-Rev
L1-ATP
L1-Theo

FIGURE 31



Rz	K_d (μM)	% Bound (max)
L1	0.16	16.5
L1-FMN	7.96	14.0
L1-Lys	2.13	22.7
L1-Theo	8.02	3.48
L1-Rev	0.77	19.0